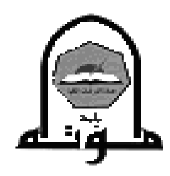


Mu’tah University
Deanship of the Graduate Studies

Synthesis, Supramolecularity and Bioactivity of New Calix[4]arene Lattice Host Molecules

By
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A Thesis Submitted to the Deanship of theGraduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry, Department of Chemistry

Mu’tah University , 2012

DEDICATION

To my parents, my sisters, my brothers, my friends, to every one who helps to produce this work and to every one who loves chemistry

Saad. S. Al-Sarhan

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First and foremost I should offer my sincere thanks and gratitude to Allah the Great from whom I receive guide and inspiration.

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Saad. Al-Sarhan

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ppm……………………………………….	Part per million
m.p……………………………………....	Melting point
NMR…………………………………….	Nuclear Magnetic Resonance
TMS…………………………………….	Tetramethylsilane
DMSO………………………………….	Dimethylsulfoxide
OTs…………………………………….	Tosyl group
FTIR…………………………………….	Fourier transform infrared
υ……………………………………….	Vibration
δ ……………………………………….	Chemical shift
br……………………………………….	Broad
sh……………………………………….	Sharp
s……………………………………….	Strong
m……………………………………….	Medium
w……………………………………….	Weak

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ABSTRACT

Synthesis, Supramolecularity and bioactivity of new Calix[4]arene lattice host molecules

Saad Al-Sarhan

Mu'tah University, December, 2012

This thesis is concerned with the preparation and characterization of new calix[4]arene derivatives through acid catalyzed condensation of resorcinol with the *p*-substituted benzaldehyde such as: *p* -chlorobenzaldehyde, *p* -bromobenzaldehyde, *p* -fluorobenzaldehyde, and *p* -methoxybenzaldehyde. The new Calix[4]arene were characterized by melting points, FTIR, ^{1}H-NMR and ^{13}C-NMR. They inhibit the growth of gram positive bacteria with brominated derivative being the most potent agent (MIC) (15.6-125 μg/ml). In addition, the solid state structure of the inclusion compounds 49 and 51 have been determined by single-crystal X-ray diffraction. The inter- and intra- molecular noncovalent supramolecular interactions involved in the crystal structure of these compounds have been carefully investigated and presented in terms of crystal engineering and supramolecular chemistry.

الملخص

التحضير والتشخيص والتركيب البلوري والنشاط الحيوي لمركبات جديدة من مشتقات كاليكس[4] ارينريسورسينول

سعد السرحان

جامعة مؤتة، 2012

تهتم هذه الأطروحة بتحضير وتشخيص مركبات جديدة من كاليكس[4]ارين الناتجة من تفاعلات تكثيف بوسط كحولي محفز حمضيا لمركب الريسورسينول مع المركبات التالية:4-كلوروبنزالدهايد ، 4- برومو بنزالدهايد، 4-فلوروبنزالدهايد، 4-ميثوكسي بنزالدهايد. تم تشخيص المركبات التي تم تحضيرها من خلال الطرق الفيزيائية المختلفة مثل قياس درجة الانصهار، الأطياف تحت الحمراء والمرئية والرنين النووي المغناطيسي للهيدروجين والكربون، كما تم تحديد التركيب البلوري لبعض المركبات الاندماجية الجديدة والتي تم تحضيرها في هذه الرسالة مثل مركبات **51** و **49** بواسطة حيود الأشعة السينية وقد تم تحديد التركيب البلوري ودراسة جميع قوى الربط الغير تساهمية الموجودة في كل شكل اندماجي. كما تم دراسة النشاطية الحيوية للمركبات المحضرة في هذه الرسالة.

CHAPTER ONE
INTRODUCTION

Background:

1.1- Supramolecular chemistry.

In recent years supramolecular chemistry established itself as one of the most active field of science (Schneider and yatsimirsky 2000). Dietrich (Dietrich, et al., 1969, 1973) and Pedersen (Pedersen, 1988), are the first pioneers in this area, they awarded the Nobel Prize for chemistry in 1986, by the development of Host-Guest complexes. Supramolecular chemistry is defined as the chemistry beyond the molecule, whereby a “supramolecule“ is a species that is held together by noncovalent interactions between two or more covalent molecules or ions also, can be described as “lego chemistry “ in which each lego brick represents as a molecular building block and these blocks are held together by intermolecular interaction that’s include electrostatic interactions, hydrogen bonding , pi-pi interactions, dispersion interactions and hydrophobic effects.(Steed, et al., 2007).

It is divided into molecular self-assembly, folding, molecular recognition, host-guest chemistry, mechanically interlocked molecular architectures, and dynamic covalent chemistry (Oshovsky, et al., 2007).

Supramolecular species was obtained from nature and specially from biological aggregates like lipid bilayers , viral capsids , DNA double helix, and the tertiary and quaternary structure of proteins.(Shakhnovich, et al., 1996),(Klug, 1983).

Nowadays the area of supramolecular chemistry stretches from molecular recognition in natural and artificial complexes to applications in new materials in biology, chemical technologies, and medicine.

1.2- Supramolecular organic chemistry.

In 1990, Wang and coworkers reported the interaction between Cyanuric acid and Melamine (Wang, et al., 1990), by solving the crystal structure from HCL solution Fig (1), in this interaction there is two dimensional hydrogen bonded molecular network based on secondary interaction between cyanuric acid and melamine.

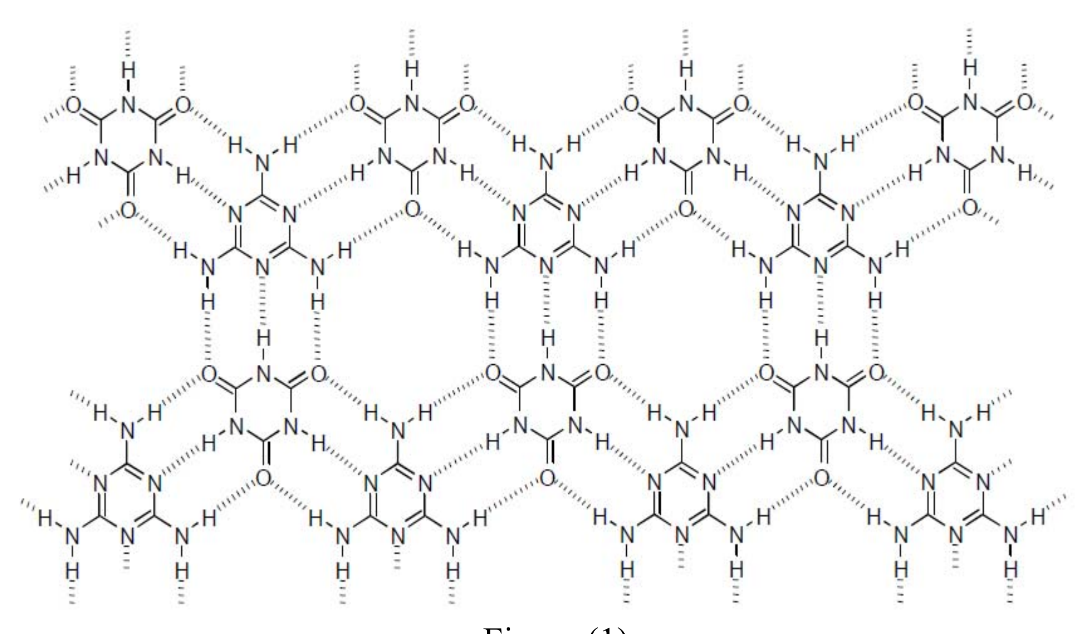


Figure (1)
The Crystal Structure of the 1:1 Complex of
Cyanuric Acid and Melamine

Also, Whitesides and coworkers successfully capitalized on this discovery by constructing roughly a globular nanostructure from cyanuric acid (**1**) and melamine derivatives (**2**) (Whitesides and Steo, 1993) to form the well-defined molecular globular structure (**3**) Fig (2).

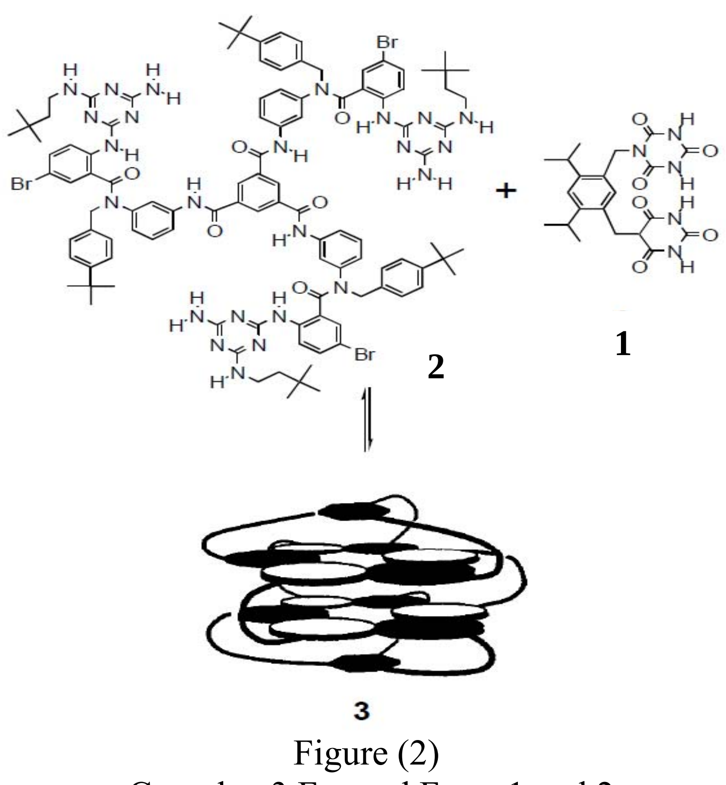
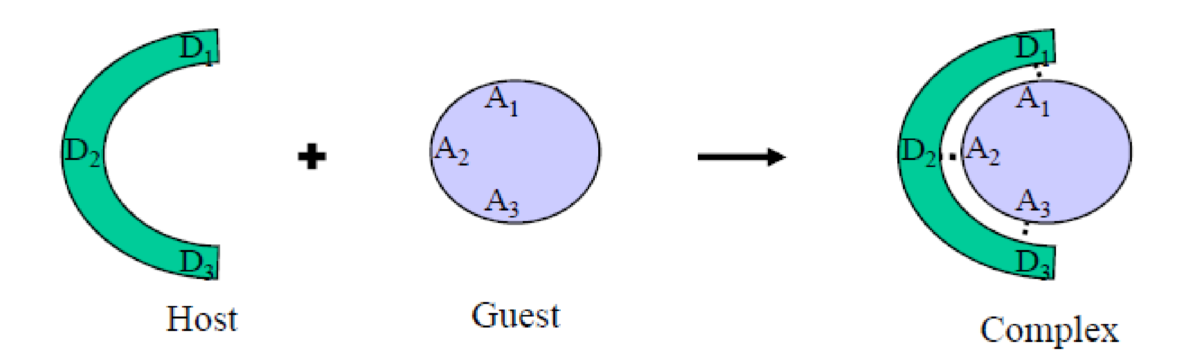


Figure (2)
Complex 3 Formed From 1 and 2

1.3- Host-Guest Supramolecular.

The host component is an organic molecules or ionic whose binding sites converge in the complex. In addition, the guest component is any molecule or ion whose binding sites diverge in the complex (scheme **1**) by non covalent interaction to forms a host-guest complexes or supramolecules, depending on the compatibility of the two or more interacting species (Cram,1986). Spherands, Calixarenes, Crown ether, Cryptands (Steed and Atwood, 2000), Resorcarenes (Sliwa, et al., 2003), Carcerands and Cavitands (Cram and Cram, 1994) have proved their ability to form host-guest complexes. Although most host molecules are organic in nature, some inorganic molecules possess concave structures capable of binding small organic molecules. Among these are zeolites and some clathrates (Steed and Atwood, 2000).



Scheme (1) - Host-Guest Complexes

In complexation supramolecular species utilize noncovalent interactions which are relatively weak in nature, the term “non covalent” covers variety of forces which are based or Permanente charge, aromatic Π character, hydrogen interaction or hydrophobic effect. Since a single non covalent bond is extremely weak, constructing a stable host-guest complex demands a host capable of utilizing non covalent interactions in summative cooperative. The extra stabilization that macrocyclic hosts provide is called “the macrocyclic effect” (Lehn, 1988).

Macrocyclic hosts offers multiple binding sites which are preorganized for interactions with the guest complex formation with a microcyclic hosts tend to be less solvated than their acyclic analogues, since the present less solvent accessible surface area. As a result, there are fewer solvent - host interactions to break in the desolvation process and a marked enthalpic gain in energy in an achieved, also binding of a guest to a macrocycle is entropically favorable due to the inherently less flexible conformation of the macrocycles (Lehn, 1988).

Now a days, Host-Guest chemistry is applied in catalysis reactions (Breslow and Campbell, 1969), Scavenging (Zhang et al., 2002), sensors (Bell and Hext, 2004) and pharmaceuticals (both drugs and delivery) (Davis and Brewster, 2004).

1.4- Hydrogen Bonds

The first definition of the hydrogen bond (H-bond) was originally proposed in 1920 by Latimer and Rodebush (Latimer and Rodebush, 1920) and finally completed and formalized by Vinogradov and Linnel in their 1971' *Hydrogen Bonding* book (Vinogradov, 1971).

A hydrogen bond exists between a functional group A-H and an atom or a group of atoms B in the same molecule (Intramolecular) or a different molecule (intermolecular) when both of atoms A & B have electronegative character, with the proton involved in the hydrogen bond being shared between the electron pairs on A & B. Specific examples that show the intramolecular and intermolecular hydrogen bonds present for the *ortho* and *para* isomers of aminobenzaldehyde are illustrated in Fig (**3**).

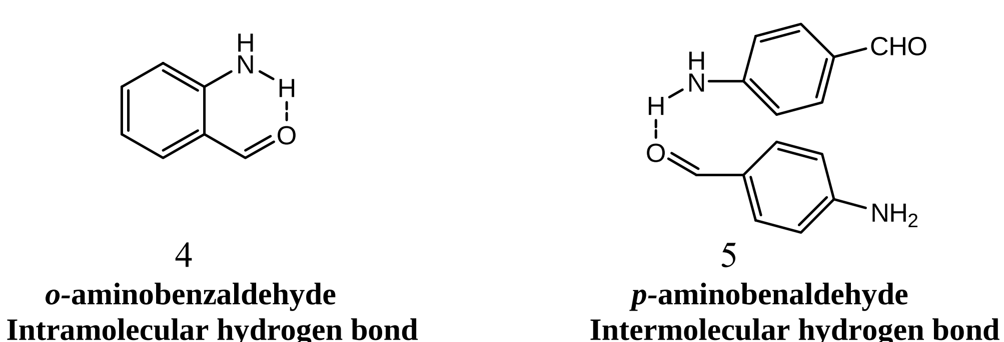


Figure (3)
Intramolecular and Intermolecular Hydrogen Bonds, Which are Produced Between the *ortho* and *Para* Isomers of Aminobenzaldehyde.

The strength of hydrogen bonds can vary from being very weak to being the strongest (and most directing) of the non-ionic intermolecular interactions. Hydrogen bonds can be detectable by spectroscopic methods as well as structural tools such as NMR, IR and neutron / X-ray diffraction (Olovsson and Jonsson 1976). Hydrogen bonding arrangements range from simple modes through to more complicated ones as illustrated in Fig (**4**). The most common hydrogen donor groups are those with acidic (polar) character (i.e. there is a difference in the electronegativity between the hydrogen atom and the adjacent atom) such as N-H, O-H, S-H, and P-H. Common acceptor groups are those with small size and high electronegativity such as, N, O, P, S, F, and Cl.

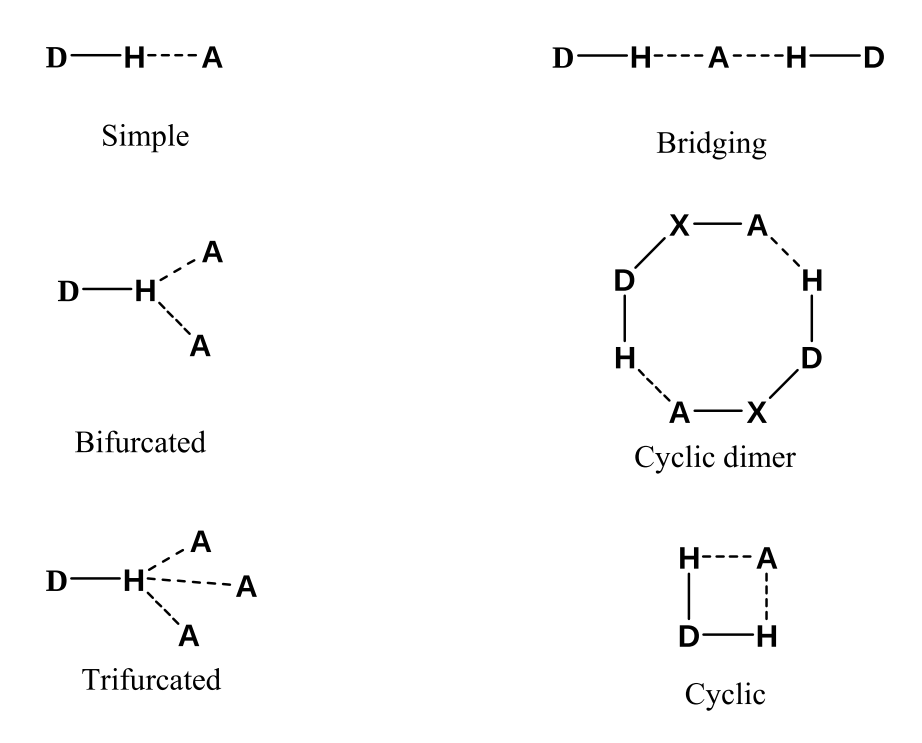


Figure (4)
Common Arrangements of Hydrogen Bond Types.

Recently, a weaker type of hydrogen bond with general formula C-H...A (A = F, O, N, Cl, Br, I) (Aakeroy and Seddon, 1993), (Desiraju, 1991), (Subramanian and Zaworotko, 1994) has been observed. This type is proved to be important in the field of supramolecular chemistry. For example, in the case of C-H...N, there are a very large number of such contacts listed in the Cambridge Structural Database (CSD) that are significantly shorter than the sum of the respective van der Waals radii (2.75 A^{o}). Contractions of this type have been used as a criterion for the existence of hydrogen bonding (Mascal, 1998).

1.5 – Calixarenes

1.5.1- History of Calixarenes

Calixarenes are the cyclic oligomers or metacyclophanes that derive from the condensation reaction of phenols and formaldehyde under basic reaction conditions. In 1944, Zinke and his coworker Erich Ziegler obtained a crystalline product while performing the condensation reaction of *p-tert*-butylphenol and formaldehyde under basic conditions. The structure was assigned as a cyclic tetramer (n = 1, Figure 5) (Zinke, et al., 1944).

	n
a	1
b	2
c	3
d	4
e	5

6

Figure (5)
General Structures of Calixarenes

In the late 1950s, experiments carried out by Cornforth and coworker (Cornforth, et al., 1973) indicates that the Zinke products were actually mixtures of two compounds. In the 1970s, Gutsche and coworkers (Gutsche, et al., 1981) reinterpreted the results of Zinke’s reaction and found that the “product” was a mixture of cyclic tetramers, hexamers and octamers. Subsequently, Gutsche’s group developed general, simple and easily reproduced procedures for synthesizing the calixarenes in good to excellent yields from a gram scale or less to many kilograms. (Gutsche, et al., 1990) This started an era of prosperous calixarene chemistry. The last 20 years saw a great development of calixarenes chemistry exploring a number of potential application fields after Gutsche’s initial suggestion that calixarenes can be considered as baskets for application in metal ion separations (Gutsche and Stoddart, 1989)

1.5.2- Synthesis of Calix[n]arenes

To date, calixarenes are synthesized by two major methods: single-step, base-induced synthesis and multi-step syntheses, each having its own pro and con. The multi step syntheses can be further classified as non-convergent stepwise synthesis and convergent stepwise synthesis.

1.5.2.1- Single-step, Base-Induced Synthesis

Developed by Gutsche and coworkers (Gutsche and Iqbal, 1990), this procedure is by far the most efficient and convenient way of preparing calix[4]arenes, calix[6]arenes and calix[8]arenes on a fairly large scale with high reproducibility scheme (2). Careful control of the reaction conditions

can give these three major *p-tert*-butylcalixarenes in 50, 85 and 63% yields, respectively (Gutsche, 1998), when different bases, as well as amounts of the base and solvents, were applied. However, neither *p*-tert-butylcalix[5]arene nor p-*tert*-butylcalix[7]arene could be obtained in comparable yields.

Scheme (2) - Single-step, base-induced Synthesis of *p*-*tert*-butylcalix[4]-, -[6]-, and -[8]arenes.

There are some drawbacks for the single-step, base-induced synthesis of calixarenes: (a) It is not as successful for *p-tert*-butylcalixarenes with odd number of aryl groups, such as p-tert-butylcalix[5]-, -[7]-, and -[9]arenes. Generally, a mixture of calixarenes is obtained and the pure product can only be isolated in very low yields. (b) It only works for those phenols with *para* alkyl substituents to give pure cyclic tetramers in reasonable yields. For instance, both *p-tert*-pentylphenol and *p-tert*-octylphenol (also known as *p*-(1,1,3,3-tetramethylbutyl)phenol) readily react with formaldehyde to give corresponding calix[4]arenes in good yields (Izatt, et al., 1985), (Bocchi, et al., 1982). Fortunately, the *tert*-butyl groups of calixarenes can be easily removed by a reverse Friedel-Crafts reaction, thus providing new positions for substitution reactions.

1.5.2.2- Multi-step syntheses

1.5.2.2.1- Non-convergent Stepwise Synthesis:

In the late 1950s, Hayes and Hunter (Hayes and Hunter 1958) synthesize p-methylcalix [4]arene by a 10-step procedure, which is shown in scheme (3) this was the first non-convergent stepwise synthesis for calixarenes. Compared with convergent syntheses, the final step of the non-convergent syntheses is characterized by the linear molecule undergoing a

ring closure reaction to form the product. Although the preparation of the final large ring molecule requires high-dilution conditions, it's still possible to obtain a single product with modest to high yield (No and Gutsche, 1982). The greatest advantage of this method is that calixarenes with larger rings, especially with odd numbered rings, can be prepared. Also, calixarenes bearing different groups at the *para*-position can be easily obtained by subsequently incorporating a different p-substituted phenol.

Scheme (3) - Hayes and Hunter's Non-convergent Stepwise Synthesis of *p*-methylcalix[4]arene.

1.5.2.2.2 Convergent Stepwise Synthesis:

To overcome the main drawbacks of the non-convergent stepwise method, i.e. the large number of reaction steps and very low overall yield, Böhmer and coworkers (Böhmer, et al., 1979), (Böhmer, et al., 1987), (Böhmer, et al., 1985), developed convergent pathways that require fewer reaction steps. This method is identified in four different ways as 3+1, 2+2, 2+1+1, and 1+1+1+1, depending on the precursors for the cyclization reaction scheme (4).

Scheme (4) - Example of 3+1 Convergent Syntheses.

1.5.3- Conformations of Calix[4]arenes and Their Characterization

1.5.3.1- Conformations of Calix[4]arenes

Calixarenes are built up by phenol and methylene units and possess several conformational isomers because of two possible rotational modes of the phenol units: the oxygen-through-the-annulus rotation and the *para*-substituent-through-the-annulus rotation Fig (6). As shown in Fig (6), the region where the *p-tert*-butyl groups are located is called the “upper rim” or “wide rim” and the region where the four hydroxyl groups lie is called the “lower rim” or “narrow rim”. Rotation of the *p-tert*-butyl groups through the upper rim is prohibited in calix[4]arenes, but is possible in calixarenes with larger rings. Hence different conformations of calix[4]arene originate from the lower rim rotation.

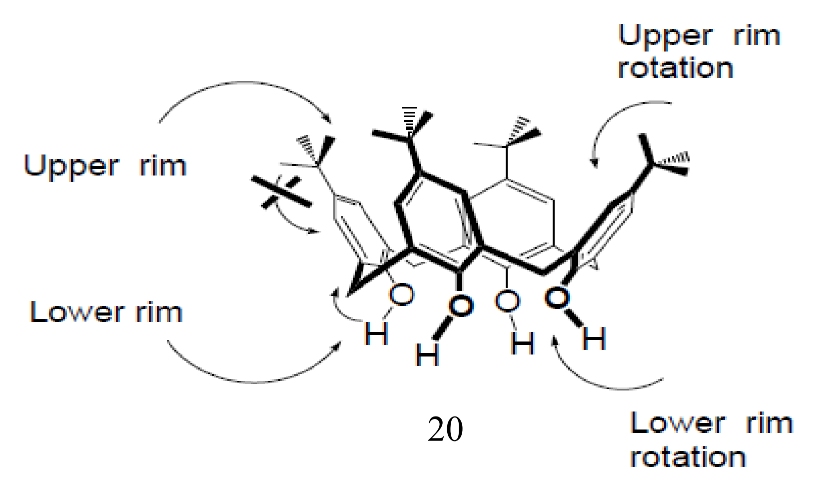


Figure (6)
Two Possible Rotational Modes of the Phenol Units.

Cornforth (Cornforth, et al., 1973) suggested that depending on the orientation of each aryl group which can project either upward or downward relative to the average plane defined by the methylene bridges. A calix[4]arene can exist in four limiting conformations: one with the aryl groups all *syn* to one another, one with three aryl groups *syn* and one anti, one with adjacent pairs of aryl groups *syn* and anti, and one with non-adjacent pairs of aryl groups *syn* and *anti*. Later Gutsche named them as

cone, partial cone, 1,2-alternate, and 1,3-alternate conformations, respectively, as depicted in Fig (7) (Gutsche, 1998).

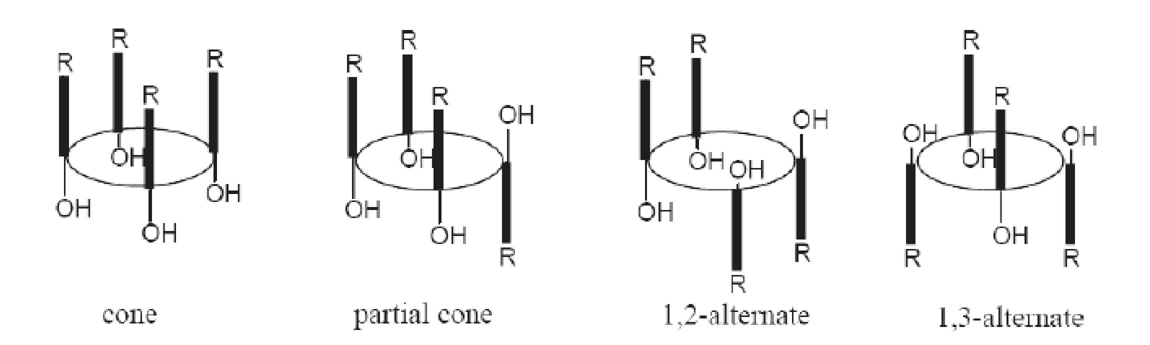


Figure (7)
Representation of the Four Main Conformations of Calix[4]arene.

The size of the substituent on the lower rim is very important for the conformations of calix[4]arenes. Experiments were performed to attach groups of different sizes to the hydroxyl group and test the mobility of the resultant calix[4]arenes. It was found that the tetramethyl, (Gutsche, et al., 1983), tetraethyl (Iwamoto, et al., 1991) and tetrakis (cyanomethyl) ethers (Guelzim et al., 1993) of calix[4]arene are all conformationally mobile, while the tetrapropyl ether (Iwamoto, et al., 1991) is conformationally rigid even at elevated temperatures. Therefore, O-substituent groups larger than ethyl locks the conformation of a calix[4]arenes into four stereoisomer. Another interesting phenomenon is that partially etherified calix[4]arenes are less flexible than the corresponding fully etherified calix[4]arenes. This is attributed to existence of intramolecular hydrogen bonding, which stabilizes the cone conformation.

The number of conformations for calixarenes with larger rings increases rapidly. If only the up-down orientation of phenolic groups is considered, calix[5]arenes, like calix[4]arenes, have only four conformers and calix[6]arenes have eight conformations, while calix[8]arenes have sixteen conformations. This number could be much higher if the flattened orientation of phenolic groups is also considered.

1.5.3.2- Characterization of Conformations of Calix[4]arenes

X-ray crystallography is a power tool for giving direct information on the orientations of molecules in the solid state. For example, the X-ray crystallographic structure of *p-tert*-butylcalix[4]arene showed that this cyclic tetramer exists in a cone conformation, with a toluene molecule in the cavity (Andreetti, et al., 1979).One of the major drawbacks of X-ray crystallography is that it requires suitable crystals, and that is a problem for many molecules, including calix[4]arene derivatives.

An easier way to identify the conformations of calix[4]arenes is NMR spectroscopy. Different conformations of calix[4]arenes can be easily identified by their ^{1}H and ^{13}C NMR spectra, typically by the patterns for the bridging methylene groups Fig (8). Although the less common 1,2-alternate conformation shows a similar pattern as the partial cone, the two conformations can still be easily distinguished from the aromatic area of the spectrum. Jaime and coworkers (Jaime, et al., 1991) introduced a very useful "rule" for correlating the ^{13}C NMR spectra of calixarenes with their conformations.

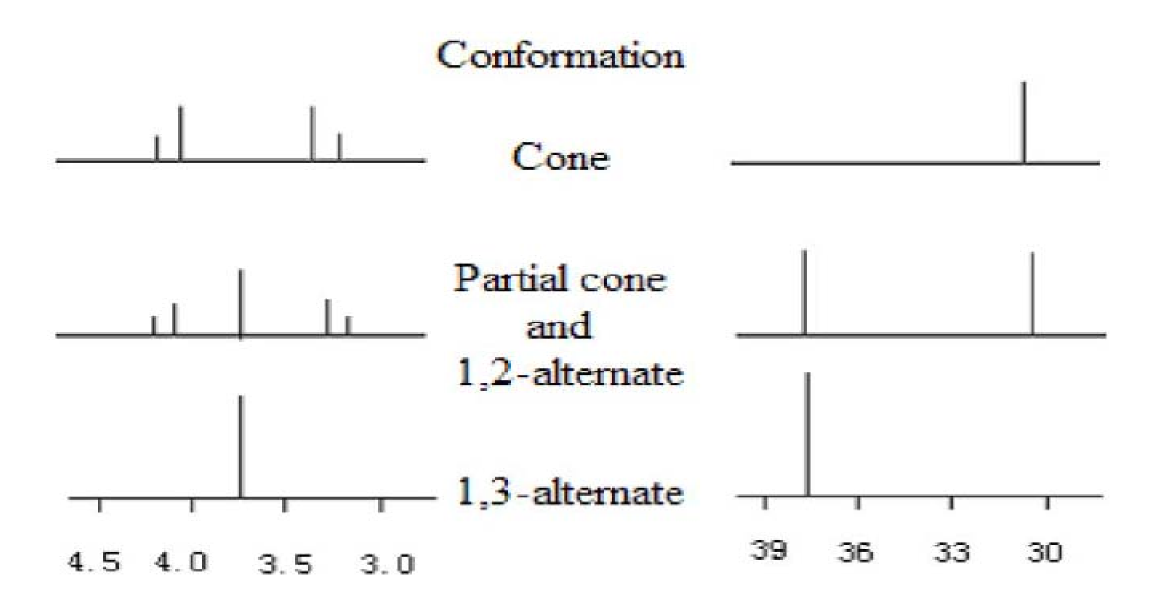


Figure (8)
^{1}H and ^{13}C NMR Patterns for Bridging Methylene Groups of Different Calix[4]arene Conformers.

When two adjacent aryl groups are in a *syn* orientation, the chemical shift for the methylene carbon is near 31 ppm and the methylene carbons will be near 37 ppm when they are *anti* to each other.

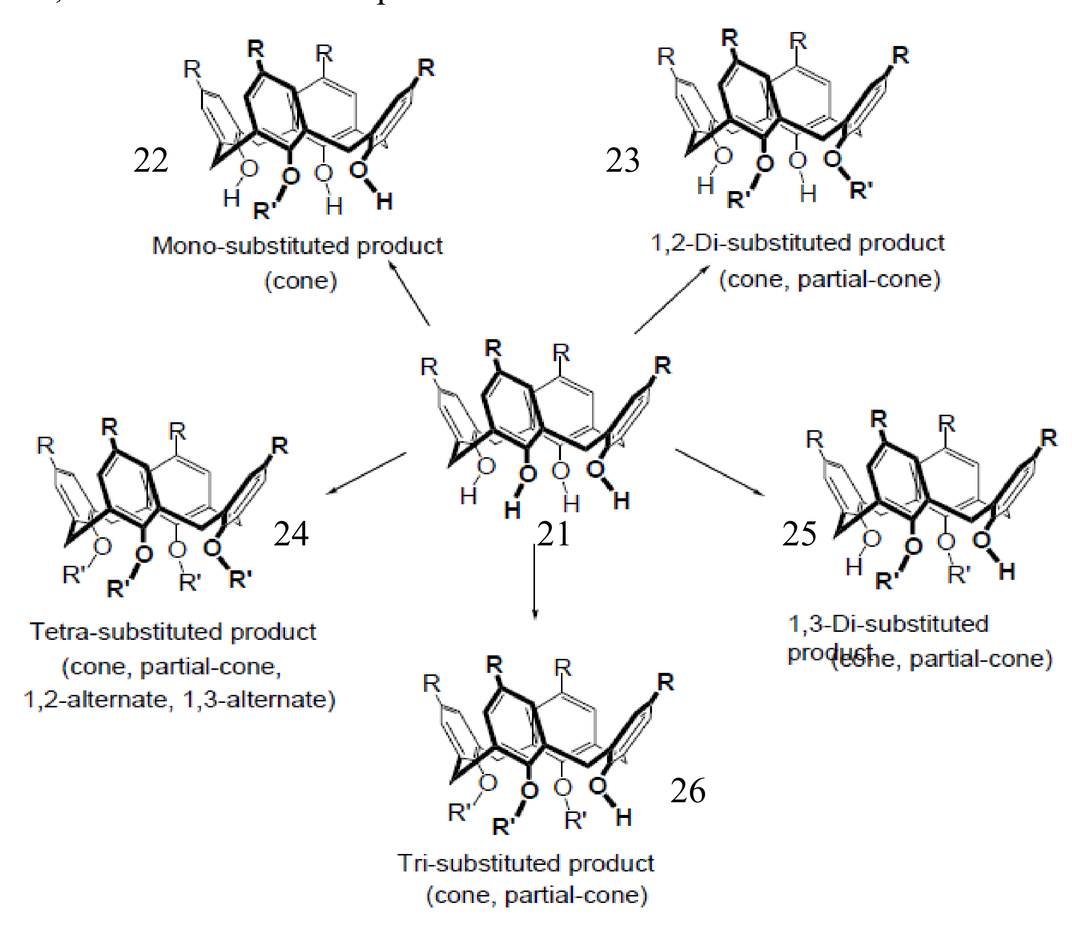
It should also be noted that, since the four conformations have different symmetry levels, the NMR patterns for other protons, such as aryl protons and protons from the *p*-substituted groups may also give important information for determination of conformations. Conformations with higher symmetry levels, such as cone and 1,3-alternate conformations, give simpler ^{1}H-NMR spectra.

1.5.4- Lower and Upper Rim Functionalization of Calix[4]arenes

Functionalization of the lower rim of calix[4]arenes by esterification and etherification reactions of the hydroxyl groups on the lower rim is the most popular and extensively studied functionalization. As shown in scheme (5), by use of appropriate reaction conditions, partial (mono-, 1,2-di- and

1,3-di-) and totally (tetra-) substituted esterification or etherification products can be obtained directly from the parent calix[4]arenes in moderate to excellent yields (Asfari, et al., 2001).

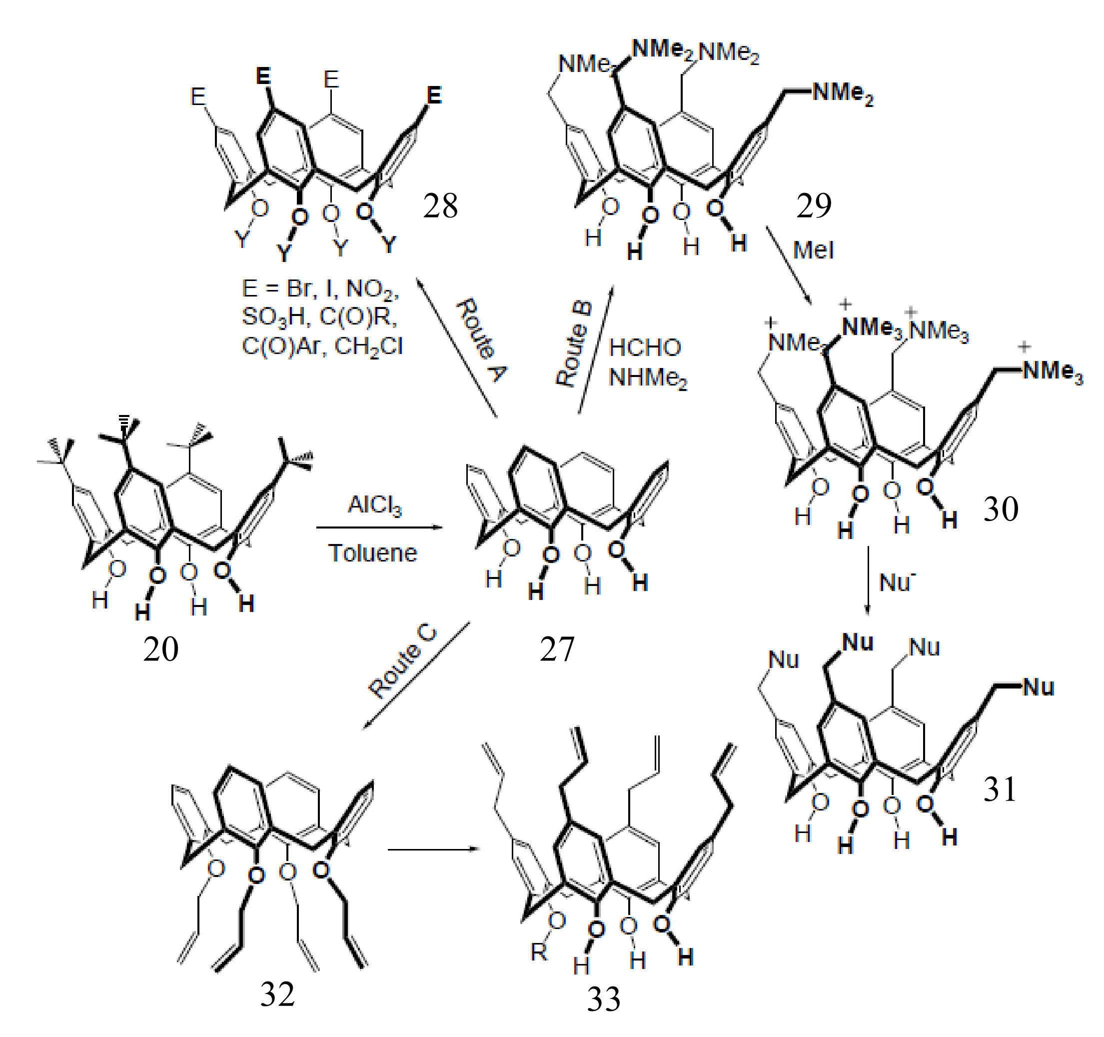
A mixture of isomers is usually obtained, if the R' group is larger than ethyl. Scheme (5) also shows observed stable conformations for the mono-, di-, tri-, and tetra-substituted products.



Scheme (5)- Partial and Total Substitution of Calix[4]arenes with Stable Conformations Shown in Parenthesis.

Upper Rim Functionalization of Calix[4]arenes, scheme (6) shows some examples (Gutsche, 1998). First, dealkylation reaction of *p*-tert-butylcalix[4]arene via a reverse Friedel-Crafts reaction gives *p*-H-calix[4]arene. Electrophilic substitution of *p*-H-calix[4]arene affords para-substituted calix[4]arenes with various para-substituents (Route A). In Route B, reaction of *p*-H-calix[4]arene with formaldehyde and dimethylamine (Mannich Reaction) gives *p*-aminomethyl-calix[4]arene, which can be converted into the corresponding quaternary ammonium compounds by reaction with MeI. Attack of the ammonium salts by a nucleophile affords *p*-Nu-methylcalix[4]arenes.

Route C utilizes the Claisen rearrangement reaction. First, *p*-H-calix[4]arene is alkaylated with allyl bromide in the presence of base to give the tetraallyl ether, which undergoes the Claisen rearrangement to give *p*-allylcalix[4]arene.



Scheme (6)- Some Examples of Upper Rim Functionlization.

1.5.5 – Calix[n]arenes in Analytical – Environmental Chemistry

These several classes of synthetic macrocyclic compound currently used as receptors (host) in supramolecular chemistry, calix[n]arenas have assumed akey rule due to their synthetic accessibility and versatility. the insertion of new functional groups onto both the upper and lower rims of the macrocycles can be easily a accomplished using common reactions, typical of the organic chemistry such functionalization reactions allow as an examples either the insertions of ancillary binding sites or the extension of the host aromatic cavity, for this reason calix[n]arene can be considered as useful building block for the synthesis of new advanced receptors.

Calix[4]arenas compounds desirable for molecular recognition and sensing applications because it has the largest available surface area for host-guest interactions .(Steed , et al.,2000) also, they have been found many applications including their use in high performance liquid chromatography (HPLC) (Zhang et al., 1997), gas purification (Atwood, et al.,2005) , storage materials (Atwood, et al,.2004) , nano filtration complexation to extract heavy metals (Nicod, et al., 1999) ,synthesis of dendrimers (Yamakawa, et al, 1998) , protein activators (Perret et al., 2005) , anticancer compounds (Harris, 1995), and drug delivery (Coleman, et al., 2003).

Ma et al, reported on several crystal structures of benzyl group in the calix[4]resorcinarene host matrixes to explore the influence of the host environmental on the properties of the guest benzyl. There is also work done to synthesize calix[4]arenes as host molecules with chiral recognition. (Ma, et al., 2003).

1.5.6 Calix[n]arenes in Biological Applications

During the last three decades, a dramatic increase in infections caused by multidrug-resistant bacteria has taken place (Soomro et al., 2012). The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. Therefore, developing new antibacterial agents that target the broadest spectrum of bacteria is required.

The calixarenes, a class of synthetic macrocycles, has attracted most of the researchers working in a wide range of fields. Due to their nontoxic nature, calixarenes have extensive applications in the biological and pharmaceutical area (Soomro et al., 2012). They have many functionalities due to their excellent organizing behaviour into rigid structures (Grare et al. 2007). They present well-defined conformational properties and cavities with molecular dimensions that enable to encapsulate guest drugs (Mokhtari and Pourabdollah, 2012). They have often been employed in recent years as carriers and spatial organizers of various kinds of active substituent (Salem, et al., 2001).

Very few reports, essentially only in the form of patents, have studied their antimicrobial properties (Grare et al. 2007). Though, most of the calixarene components have been reported for their efficacy against few microorganism species (i.e. bacteria, fungi and viruses) (Cornforth. et al., 1955) (Casnati, et al., 1996) (Mourer, et al., 2006), some calixarenes have shown interesting activities against cancerous cells (Mourer et al., 2010) (Mokhtari and Pourabdollah, 2012).

They may be useful as drugs in the treatment of tuberculosis and mycobacterioses (Hart, et al., 1996) and calixarene based antibiotic mimics were active against bacteria, fungi, cancerous cells and viruses (Fujiwara, et al., 1998) (Harris, 1995) (Korchowiec, et al., 2007). Moreover, they were anti-thrombosis and fibrosic diseases (Hwang, et al., 1994) (Da Silva et al., 2004).

When the hydrophobic calixarene derivatives are designed, the lack of solubility in biological media made them unsuitable for in vitro standard evaluation as antimicrobial agents. Gaining hydro-solubility leads to access the biological activities. Thus, researchers are developing the synthetic strategies leading to a water-soluble analogue via introduction of hydrophilic groups at the upper and lower rims of the calixarene scaffold (Mokhtari and Pourabdollah, 2012).

Ungaro et al. studied macrobicyclic peptido calixarenes that linked with the D-alanyl-D-alanine residue of the peptidoglycan as vancomycin mimics (Casnati et al., 1996). Coleman et al (2003) reported that p-sulfonatocalix[n]arenes showed no hemolytic effect or toxicity to human erythrocytes and did not provoke immune reactions compared to cyclodextrins. He and his coworker showed that amphiphilic calixarenes are able to form highly stable monodisperse nanoparticles in water and thus could be used in pharmaceuticals drug delivery (Houel, et al., 2002) (Shahgaldian, et al., 2003).

Grare et al. (2007) observed that para-guanidinoethylcalix[4]arene showed a broad antibacterial spectrum, both on Gram-positive and on Gram-negative bacteria compared with the inactive para-guanidinoethylphenol monomer and the calixarene. Soomro et al. (2012) assessed the bioactivity of the water soluble calixarene, 5,11,17,28-tetrakismorpholinomethyl-25,26,27,28-tetrahydroxycalix[4]arene and proved that they were active against both Gram negative and Gram positive bacteria with a promising MIC-value.

Mourer et al. (2010) deduce that anionic water soluble calixarenes possess antiviral activity. Amr et al. (2002) evaluate the antimicrobial activity of several synthesized macrocyclic pyridine derivatives and showed that macrocyclic calixarene hydrazones have higher antimicrobial activity compared with used antibiotic references.

1.6 Review

1.6.1 Complexation of Metal Ions by Calix[4]arenes

Several methods were repeated to prepare different derivatives of calix[4]arene, Qazi ,et.al (2010) ; synthesized calix **36** by the reaction of *p-tert*-butyl calix[4]arene **34** with phenol-$AlCl_3$/in dry Toluene solvent to form **35** , then reacts **35** with $H_{11}C_5N$-HCHO/in acetic acid:THF solvent to obtain calix **36** , scheme (7).

34 35 36

Scheme (7) - Synthesis of Ligand 36: (a) H_5C_6OH, $AlCl_3$/ (dry) Toluene (b) $H_{11}C_5N$-HCHO / Acetic acid: THF.

It has been found that the calix **36** , act as ligand has are markable complexation ability for all selected transition metal ions such (Cd^{+2} , Cu^{+2} , Ni^{+2} , Co^{+2} , Pb^{+2} , Hg^{+2}) with exceptionally high affinity for Hg^{+2} ions also, by applying method of continuous variation such that Job's method , has been found strong complexation behavior of ligand **36** for Hg^{+2} ion.

1.6.2– lattice Inclusion System of a Ternary Calix[4]arene.

Alshahateet and coworkers (Alshahateet, 2010) (Alshahateet et al., 2007), Synthesized C-phenylcalix[4]pyrogallolarene host lattice **39** as shown in scheme (8), through the acid catalyzed condensation reaction of benzaldehyde **37** with pyrogallol **38** at different reactions temperatures, they found that this type of ternary calix[4]arenes lattice can be exploited as potential cocrystal former with active pharmaceutical ingredient.

37 38

Conc. HCl, EtOH

39

Scheme (8) - Synthesis of *C*-Phenylcalix[4]Pyrogallolarene

1.6.3 – Calix[4]arene in Selective Extractions.

Osipov (Osipov et al., 2008) synthesized the fluorus calix[4]arenes (**41a-b**) by O-alkylation of calix[4]arene **40** , with 3-(prefluoro-hexyl) propyliodide and 3-(prefluoro-octyl)propyliodide respectively in dimethyl formamide solvent scheme(**9**).

$AlCl_3$ PhOH PhMe; NaH I-R_{hf} DMF

40

41a:R_{hf}=$(CH_2)_3C_6F_{13}$
41b:R_{hf}=$(CH_2)_3C_8F_{17}$

Scheme (9)-Preparation of 41a and 41b

They found the solubility of **41a**, and **41b** in variety of organic and fluorus solvent table **1**, as with many calixarenes **41b** was highly soluble in chloroform and in fluorophilic solvents such as THF and diethyl ether.

Table (1)
Solubility of 41a and 41b in Flourous Solvents

[**3a**]\Solvent	FC-72[b]	FC-75[b]	FC-77	HFE-7100	HFE-7500	F-626
1 mM	–	–	–	+	+	+
2 mM	–	–	–	+	+	+
5 mM	–	–	–	+	+	+
10 mM	–	–	–	+	+	+
[**3b**]\Solvent	FC-72	FC-75	FC-77	HFE-7100	HFE-7500	F-626
1 mM	+	+	+	+	+	+
2 mM	–	–	–	+	+	+
5 mM	–	–	–	+	+	+
10 mM	–	–	–	+	+	+

Similarly, 38b was soluble in fluorous solvents, Fe-72 (perfluorohexanes), FC-77 (perfluorooctanes), and HFE-7100 (methylnano-fluorobutyl ether). HFE-7500 (ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethylhexane) and F-626 (1H,1H,2H,2H-perflourooctyl-1,3-dimethylbutyl ether) at 1mM concentration (Osipov, et al., 2008).

1.6.4 - Asymmetric Calix[4]arene Quinone as Intermediate.

Taghvaei– Gaujali (Taghvaei-G, et al., 2002), was described a simple method for the preparation of asymmetrically, meta-substituted calixarene by direct interaction of a iodine atom at the meta position of one aryl unit scheme (**10**)

This synthesis was fisted by oxidation of rigidly cone conformation of calix[4]arene triether **42** by HIO_3/I_2 affords mixture to form of quinone **43** and its iodo substituted derivative **44** (in 3:1) ratio , then reduction of **43** ,**44** to produce 45 ,**46** in quantitative yield . Products **39** and **40** could be used to build up the chiral calix[4]arenes hosts that are useful as enzyme mimics (Taghvaei- G .et al., 2002).

Scheme (10) Asymmetric Calix[4]arene Quinone as Intermediate

1.7 – Aim of the study:

1- Design, synthesis and characterization of new family of potential host molecules (calix[4]arenes).
2- Screening the ability of the prepared derivatives to form host-guest complexes.
3- Studding the host-guest complex(s) in term of supramolecular chemistry and crystal engineering.
4- Testing the antimicrobial activity of the prepared derivatives as well as their host-guest complexes (if any).

CHAPTER TWO
EXPERIMENTAL

2.1 Materials

The solvents used were analytical reagent grade and were used as purchased. The compound resorcinol was purchased from BDH chemicals Ltd., Poole, England. The compounds 4-clorobezaldehyde, 4-bromobezaldehyde, 4-florobezaldehyde, ethanol, ethylene glycol, cyclopentanol and hydrochloric acid were purchased from Aldrich chemical company. The compound 4-methoxybenzaldehyde (4-Anisaldehyd) was obtained from Fluka.

2.2 Physical measurements

Melting points were measured on a Stuart scientific melting point apparatus in open capillary tubes. The infrared spectra were recorded over the range 4000-500 cm^{-1}, on a Maltson 5000 FTIR spectrophotometer; potassium bromide pellets were used. The ^{1}H-NMR and ^{13}C-NMR experiments were conducted on a Bruker 500 MHz with TMS as the internal standard at the University of Jordan. Chemical shifts were referenced to *TMS* as the internal standard and deuterated dimethylsulfoxide (DMSO-d_6) as the solvent.

Synthesis of some Calix[4]arene compounds

HCl / EtOH

47a-d   48   49-52

Compd NO.	49	50	51	52
R	Cl	Br	F	$-OCH_3$

Scheme (11): Synthesis of Calix[4]arene compounds (**49-52**).

2.3 General procedure:

A solution of 4-halobezaldehyde (45.4 mmol) (47a-d) in 15 ml ethanol was added dropwise to an ice-cooled solution of resorcinol (5.00 g; 45.4 mmol) (49-52) in a mixture of 15ml ethanol and catalytic amount (3 ml) of concentrated HCl. The reaction mixture was then stirred at either room temperature or refluxed for 24 h. The selected IR, ^{1}H-NMR and ^{13}C-NMR data are presented in Tables **3**, **4**, **5**, respectively for all prepared compounds.

2.3.1 Preparation of $C_{52}H_{36}Cl_4O_8$, (49)

This compound was prepared from 4-clorobezaldehyde (6.386 g; 45.4 mmol) (47a) and resorcinol (5.00 g; 45.4 mmol) (48); after 30 min a green solid precipitate (49) was formed, following the general procedure mentioned. The yield was 4.88 g (11.5 %). m.p = 100 ^{o}C (dec).

49

$C_{52}H_{36}Cl_4O_8$

Exact Mass: 928.1164
Mol. Wt.: 930.6494

2.3.2 Preparation of $C_{52}H_{36}Br_4O_8$, (50)

This compound was prepared from 4-bromobezaldehyde (8.39 g; 45.4 mmol) (47b) and resorcinol (5.00 g; 45.4 mmol) (48) after 60 min orange solid precipitate (50) was formed, following the general procedure mentioned. (Linda, et al. 1989) The yield was 5.28 g (10.5 %). m.p = 90 ^{o}C (dec).

50

$C_{52}H_{36}Br_4O_8$

Exact Mass: 1103.9144
Mol. Wt.: 1108.4534

2.3.3 Preparation of $C_{52}H_{36}F_4O_8$, (51)

This compound was prepared from 4-fluorobezaldehyde (5.63 g; 45.4 mmol) (47c) and resorcinol (5.00 g; 45.4 mmol) (48), after 30 min a pale brown precipitate (51) was formed, following the general procedure mentioned. The yield was 3.90 g (10 %). m.p = 100 ^{o}C (dec).

51

$C_{52}H_{36}F_4O_8$

Exact Mass: 864.2346
Mol. Wt.: 864.8311

2.3.4 Preparation of $C_{56}H_{48}O_{12}$, (52)

This compound was prepared from 4-methoxybezaldehyde (6.18 g; 45.4 mmol) (47d) and resorcinol (5.00 g; 45.4 mmol) (48), after 30 min a chestnut precipitate (52) was formed, following the general procedure mentioned. (Utomo, et al. 2011) (Linda, et al. 1989) The yield was 5.10 g (12.3 %). m.p = 120 ^{o}C (dec).

52

$C_{56}H_{48}O_{12}$

Exact Mass: 912.3146
Mol. Wt.: 912.9731

Table (2)

Summery of some physical properties for the prepared compounds (49-52)

Compound NO.	Molecular formula	Physical properties		
		M.p.°C (Dec.)	Color of product	% yield
49	$C_{52}H_{36}Cl_4O_8$	100	green	11.5
50	$C_{52}H_{36}Br_4O_8$	90	orange	10.5
51	$C_{52}H_{36}F_4O_8$	100	pale brown	10
52	$C_{56}H_{48}O_{12}$	120	chestnut	12.3

Table (3)
Infrared (IR) bands for different solvated solvents of a certain functional groups in cm^{-1} for (49-52) compounds (KBr /pellets)

Compound	Solvent / Vibration				Assignment
	Free solvent	Ethanol	Ethylene glycol	Cyclopentanol	
49	3435b	3403b	3420b	3428b	υ_{O-H}, phenolic
	1620m,1510w, 1489m	1618s,1510s, 1489s	1618m,1524w 1489m	1618m,1508w, 1489m	$\upsilon_{C=C}$ aromatic
	1429m	1429s	1429m	1431m	υ_{C-H} alkane
	1209m,1078m 1014w	1242w, 1209s, 1078s, 1014m	1207m,1078s, 1037w	1209m, 1078m, 1015w	υ_{C-O} phenolic
	552m	552m	552m	552m	υ_{C-Cl}
50	3441b	3441b		3447b	υ_{O-H}, phenolic
	1618m,1508m ,1485m	1618m,1510m, 1485m		1620m,1510m, 1485m	$\upsilon_{C=C}$ aromatic
	1429s, 1402w	1431s	-----------------	1431m, 1402w	υ_{C-H} alkane
	1240w,1209m, 1078s, 1011m	1275w, 1240m, 1209s, 1078s		1238w, 1209m, 1078s	υ_{C-O} phenolic
	550m	550m		550m	υ_{C-Br}
51	3412b	3410b	3403b	3435b	υ_{O-H}, phenolic
	1605m, 1508s	1604s, 1510s	1605m, 1508s	1605m, 1508s	$\upsilon_{C=C}$ aromatic
	1431m,	1431s	1431m,	1431m,	υ_{C-H} alkane
	1213m,1159m , 1076m	1213s, 1159m, 1076s,	1275w,1217s, 1157m,1076m	1211m, 1157w, 1078m	υ_{C-O} phenolic
	553m	554m	553m	553m	υ_{C-F}
52	3408b	3403b	3401b	3412b	υ_{O-H}, phenolic
	1609m, 1510s	1607m, 1510s	1609m, 1512s	1609m, 1512s	$\upsilon_{C=C}$ aromatic
	1429m	1429w	1476w, 1427w	1429m	υ_{C-H} alkane
	1246m,1208sh 1180m,1147w, 1076m, 1032w	1244m, 1181m, 1148w, 1078m	1258w,1208w 1182w,1080w	1246m, 1180m, 1048w, 1078m	υ_{C-O} phenolic

Abbreviations: s, strong; **m**, medium; **b**, broad; **w**, weak; **sh**, sharp

Table (4)

^{1}H NMR Chemical shifts (δ) for (49-52) compounds [a]

Compound	Chemical shifts: δ in ppm
49 ($C_{52}H_{36}Cl_4O_8$)	δ= 8.69 (br, 8H, O*H*), 7.05(d, ^3J = 8.5Hz, 8H, H_d), 6.63 (d, ^3J = 8.5Hz, 8H, H_c), 6.19 (br, 4H, H_b), 5.62 (s, 4H, H-benzylic).
50 ($C_{52}H_{36}Br_4O_8$)	δ= 8.70 (s, 8H, O*H*), 7.19(d, ^3J = 8.5Hz, 8H, H_d), 6.58 (d, ^3J = 8.5Hz, 8H, H_c), 6.18 (br, 4H, H_b), 5.61 (s, 4H, H-benzylic).
51 ($C_{52}H_{36}F_4O_8$)	δ= 8.63 (s, 8H, O*H*), 6.80(t, $^3J_{H-H}$ = 8.5Hz, 8H, H_d), 6.65 (dd, $^3J_{H-F}$ = 8.5Hz, $^3J_{H-H}$ = 8.4Hz, 8H, H_c), 6.17 (br, 4H, H_b), 5.63 (s, 4H, H-benzylic).
52 ($C_{56}H_{48}O_{12}$)	δ= 8.42 (s, 8H, O*H*), 7.19(d, ^3J = 8.4Hz, 8H, H_d), 6.58 (d, ^3J = 8.4Hz, 8H, H_c), 6.24 (br, 4H, H_b), 5.61 (s, 4H, H-benzylic); 3.72 (s, 3H, -OCH_3).

[a] ^{1}H-NMR were obtained in DMSO-d_6 with TMS as internal standard (500 MHz).
d, doublet; **dd**, doublet of doublet; **br**, broad; **s**, singlet; **t,** triplet; **m**, multiplet.

Table (5)
^{13}C- NMR Chemical shifts (δ) for (49-52) Compounds [a]

Compound	**Chemical shifts: δ in ppm**
49 ($C_{52}H_{36}Cl_4O_8$)	41.4(C - 5), 102.5 (C – 1);120.3 (C – 3); 127.5 (C – 8); 129.9 (C – 6); 130.4 (C – 7); 145.3 (C – 2); 155.3 (C – 9).
50 ($C_{52}H_{36}Br_4O_8$)	41.4(C - 5), 102.6 (C – 1);118.3 (C – 3); 120.2 (C – 4); 128.6 (C – 8); 130.5 (C – 6); 130.9 (C – 7); 145.8 (C – 2); 153.7 (C – 9).
51 ($C_{52}H_{36}F_4O_8$)	41.3(C - 5), 102.6 (C – 1); 114.1 (d, $^{2}J_{C-F}$ = 21Hz C – 8); 120.8 (C – 3); 130.3 (d, $^{3}J_{C-F}$ = 8Hz C – 7); 142.3 (C – 6); 153.2 (C – 2); 160.5 (d, $^{1}J_{C-F}$ = 239 Hz, C – 9).
52 ($C_{56}H_{48}O_{12}$)	41.7(C - 5), 55.1 (-O*C*H_3); 102.5 (C – 1); 113.0 (C – 8); 122.0 (C – 3); 129.9 (C – 7); 139.1 (C – 6); 153.8 (C – 2); 157.9 (C – 9).

[a] ^{13}C-NMR data in DMSO-d_6 as solvent and TMS as internal standard (125 MHz).

2.4 In Vitro Antimicrobial Activity

The antibacterial activity of was determined by measuring the inhibition zones in agar diffusion test and calculating the minimum inhibitory concentration (MIC) by serial dilution assay as described earlier (Alshahateet et al., 2011). The test microorganisms used in this study were *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (ATCC 25922), *Enterobacter aerogenes* (ATCC 13048), and *Micrococcus luteus* (ATCC 10240).

Briefly, Muller-Hinton agar plates seeded with 10^6 cell/mL of test bacterial strains were used for agar diffusion test. Six millimeter sterile filter paper discs impregnated with four different concentrations (0.1, 0.25, 0.5, 1 mg/disc) of the test samples were placed on the surface of the plates. All the plates were incubated at 37 °C for 24–48 h. Antimicrobial activity was calculated by measuring the diameter of the inhibition zones. Minimum inhibitory concentration (MIC) was determined using serially dilutions of test samples and positive control (Chloramphenicol) starting from a final concentration of 1 mg/mL and 100 µg/mL, respectively. Test samples (100 µL) were diluted serially in 96 well plates and each microbial strain suspension (100 µL of 2×10^6 cell/ml) was added in each well. All prepared cultures were incubated at 37 °C for 24 h. The MIC was determined as the minimum concentration of test sample that inhibits growth of microorganism and the OD_{600nm} of the culture is near to or equal zero. The experiment was performed in triplicate.

2.5 Solution and Refinement of the Crystal Structures

Reflection data were measured at 223(2) K on a Bruker SMART Apex-1000 diffractometer equipped with a CCD detector and Mo-Kα sealed tube at National University of Singapore (NUS, Singapore). Smart was used for collecting frame data, indexing reflection, determination of lattice parameters, integration of intensity of reflections and scaling. SADABS was used for absorption correction and SHELXTL for space group, structure determination, and least-square refinements on F^2.

All non-hydrogen atoms were assigned anisotropic displacement parameters in the refinement. All hydrogen atoms were added at calculated positions and refined using a ridging model.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1. Results

The synthesized calixarenes (49, 50, 51, 52) were prepared via condensation reaction of 4-clorobenzaldehyde (47a), 4-bromobenzaldehyde (47b), 4-florobenzaldehyde (47c) or 4-methoxybenzaldehyde (47d) reacted with resorcinol in ethanolic solution in the presence of a catalytic amount of acid as shown in scheme (11) according to the literature procedure (Firdaus et al., 2008) (Cram. et al., 1998), (Linda, et al. 1989) . The detailed mechanistic aspects are shown in scheme (12). (Weinelt and Schneider, 1991) (Utomo, et al. 2011)

Scheme 12: preparation mechanism of Calix **49- 52**

Some physical properties (color and melting point) are listed in Table **2**, also FT-IR absorption bands along with their assignments are presented in Table **3**. The important bands of the prepared calixarene were assigned by comparing them with those that reported (Gutsche, et al., 1990) (Izatt , et al., 1985) (Bocchi, et al., 1982) (Böhmer, et al., 1987) (Iwamoto, et al., 1991), (Iwamoto, et al., 1991) (Guelzim, et al., 1993) and (Hebbink, et al., 2001). ^{1}H-NMR and ^{13}C-NMR measurements were carried out for calixarenes 49-52 (Table **4** .and Table **5**) respectively, the structure of compounds (49-52) are in accordance with the IR and NMR spectra.

The test microorganisms used in this study were Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 43300, Micrococcus luteus ATCC 10240. Antibacterial activity were carried out and determined as described by Abu-Shanab and coworker (Abu-Shanab, et al., 2004) with some modification were presented in table **6** and **7** respectively.

3.2. Discussion

3.2.1 Synthesis

The calixarene were prepared by condensation reaction of ice-cooled solution of resorcinol in a mixture of ethanol and catalytic amount of concentrated HCl with four different of *para*-substituted benzaldehyde solution, 4-clorobezaldehyde, 4-bromobezaldehyde, 4-florobezaldehyde, and 4-methoxybenzaldehyde, in a 1:1 molar ratio (Scheme **11**). The prepared calixarenes were characterized by FTIR (Table **3**), ^{1}H-NMR (Table **4**) and ^{13}C-NMR (Table **5**).

3.2.2 Infrared measurements

The characteristic infrared bands for calixarene are shown in Table **3**. The IR spectra of compound 49- 52 shows a υ(C-H, aliphatic) absorption band in the range of 1476-1429 cm^{-1} and display a broad bands in the range of 3447-3401 cm^{-1} for phenolic O-H bond. The (-C=C, aromatic) display in the range of 1605-1620 cm^{-1}, in addition to absorption band in the range of 550-553 cm^{-1} due to carbon- halogen (Cl, Br, F) bond and 1250 cm^{-1} for the O-CH_3 in 52 compound. The absence of a υ(C=O) band in the range of 1740-1720 cm^{-1} confirmed that the benzaldehyde derivatives are reacted. (Kusumaningsih, et al.2010) (Linda, et al. 1989) (Jumina, et al. 2007) (Maulidan , et al. 2008) (Morita, et al. 1992). Figure (9,10) shows the infrared spectra of the calixarene (51, 52).

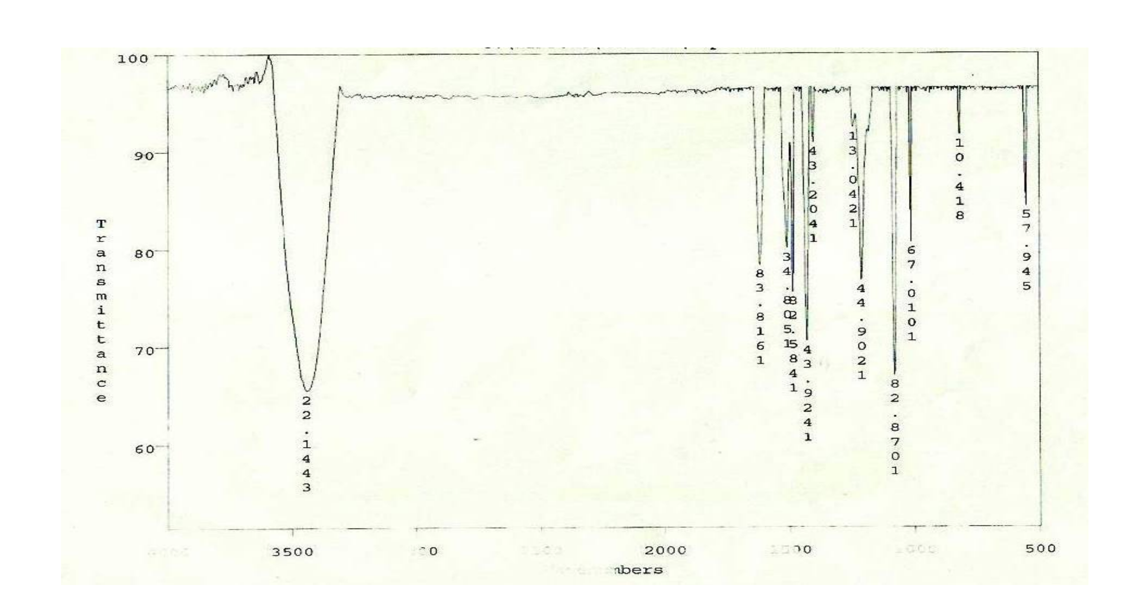


Figure 9

FTIR spectra of $\mathbf{C_{52}H_{36}F_4O_8}$, **(51)**

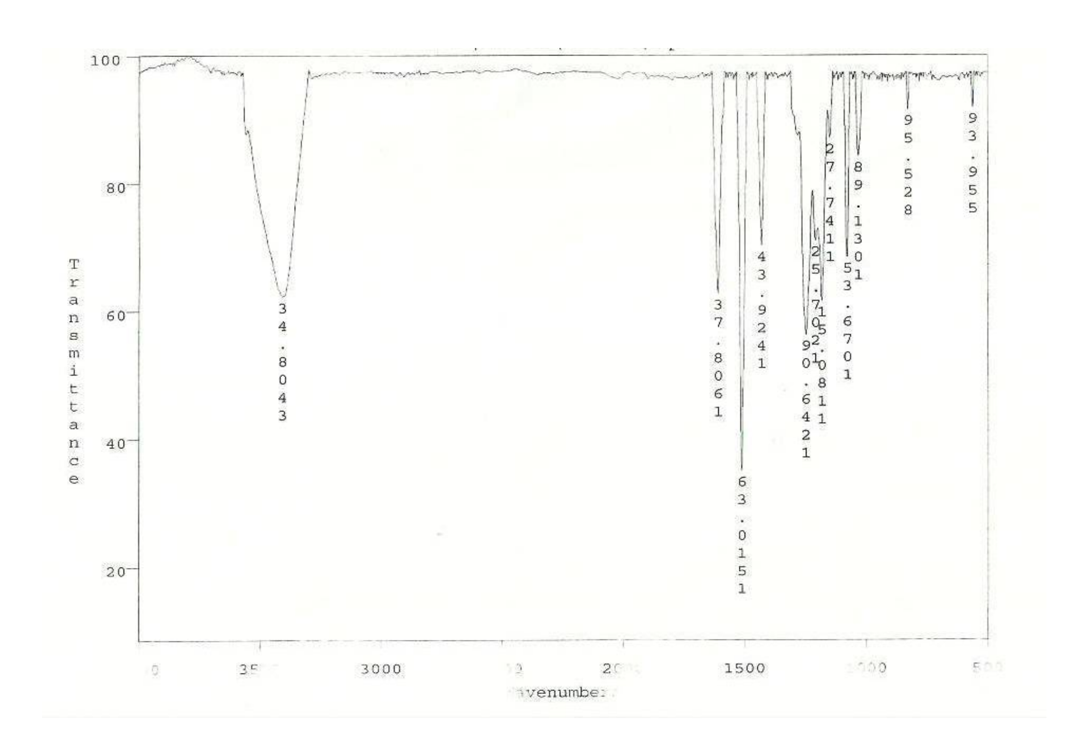


Figure 10

FTIR spectra of $\mathbf{C_{56}H_{48}O_{12}}$, **(52)**

3.2.3 NMR spectra

The ^{1}H-NMR and ^{13}C-NMR spectral data for compounds are listed in Table **4** and Table **5**, respectively. The ^{1}H-NMR spectra for the prepared calixarene gives signal corresponding to the aromatic proton (belonging to the benzaldehyde ring) in the range 5.50- 7.30 ppm as shown in the representative spectrum of the title compound (49) shown below.

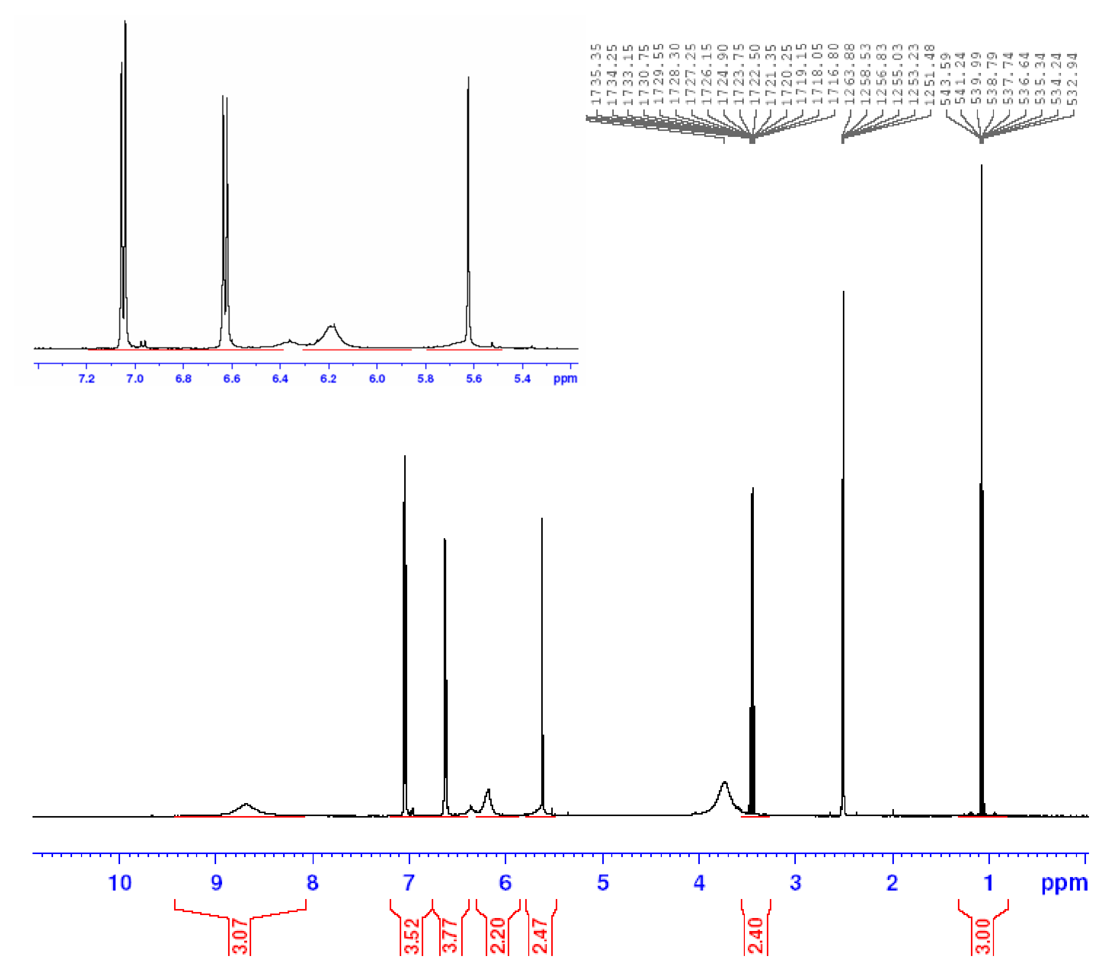
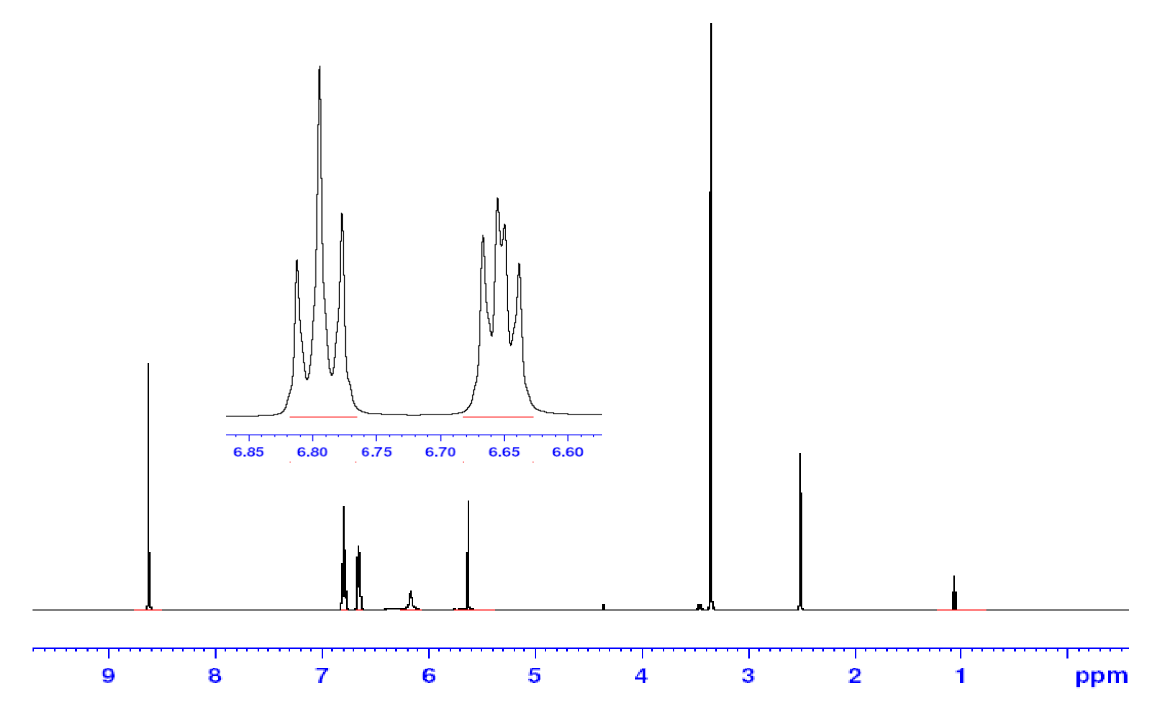


Figure (11)
^{1}H-NMR spectrum for 49 ($C_{52}H_{36}Cl_4O_8$) compound
obtained in DMSO-d_6 with TMS as internal standard (500 MHz).

The methylene protons resonates as a singlet in the range of 5.60-5.63 ppm , while the phenolic protone resonates in the range of 8.41-8.70 ppm. The other protons belonging to resorcinol ring are readily organized in table **4**.
The H-c and H-d protons resonates as a doublets in the range of 6.58-7.19 ppm , (^{3}J = 8.5Hz) due to spin-spin coupling with the *ortho* H- atom at C-7 and C-8 atoms.

The ^{1}H-NMR spectra for compound 51 display the effect of fluorine atom on the splliting of adjacent protons ($^{3}J_{H-F}$ = 8.5 Hz) as shown below.



The [13]C-NMR spectral data support the formation of calixarene through absente of aldehydes signals in the region 191 ppm and Presence of methylene bridge (CH-aliphatic) signals in the region 41.4-41.2 ppm. The aromatic carbon are resonates in the region of 102.5-161.4 ppm. Representative [13]C-NMR spectra for 49 compound is shown below. The other ring carbons are mentioned in table 5.

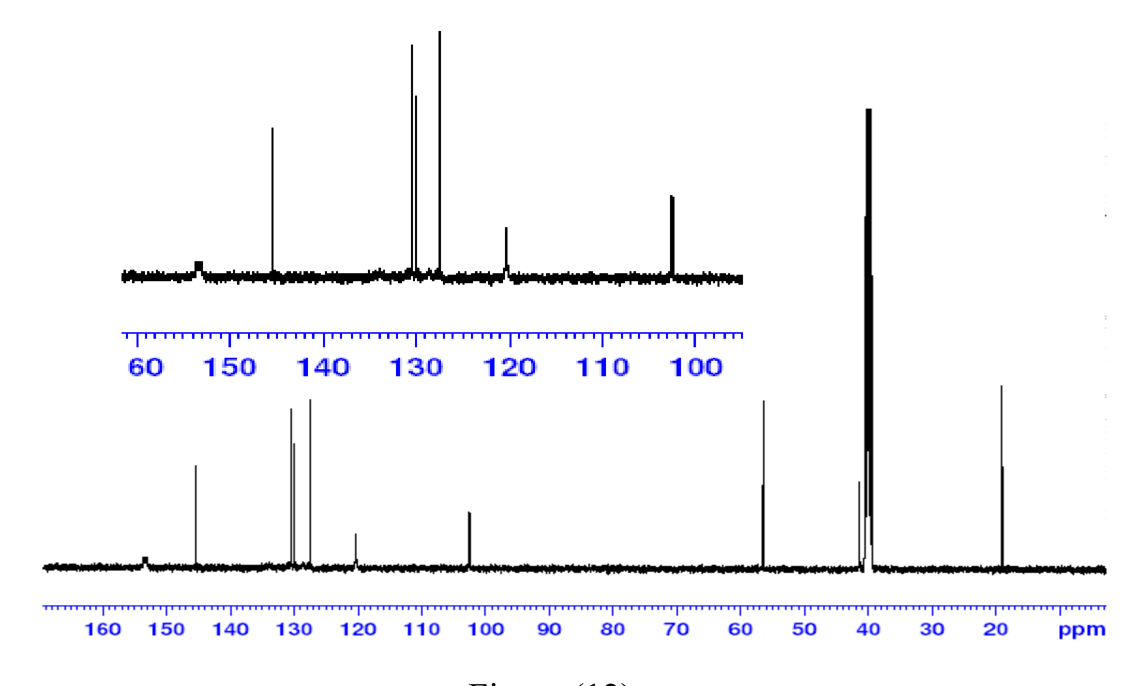


Figure (12)
^{13}C-NMR spectra for 49 ($C_{52}H_{36}Cl_4O_8$) compound in DMSO-d_6 as solvent and TMS as internal standard (125 MHz).

The ^{13}C- NMR spectrum for the C – 9, C - 8 and C – 7 carbons are distinguishable due to spin – spin coupling with the fluorine atom at C – 9 position. Thus, C – 9 resonates at 160.5 ($^{1}J_{C\text{-}F}$ = 239 Hz); C – 8 resonates at 114.1 ($^{2}J_{C\text{-}F}$ = 21 Hz) and C – 7 resonates at 130.0 ($^{3}J_{C\text{-}F}$ = 8 Hz) Figure (13).

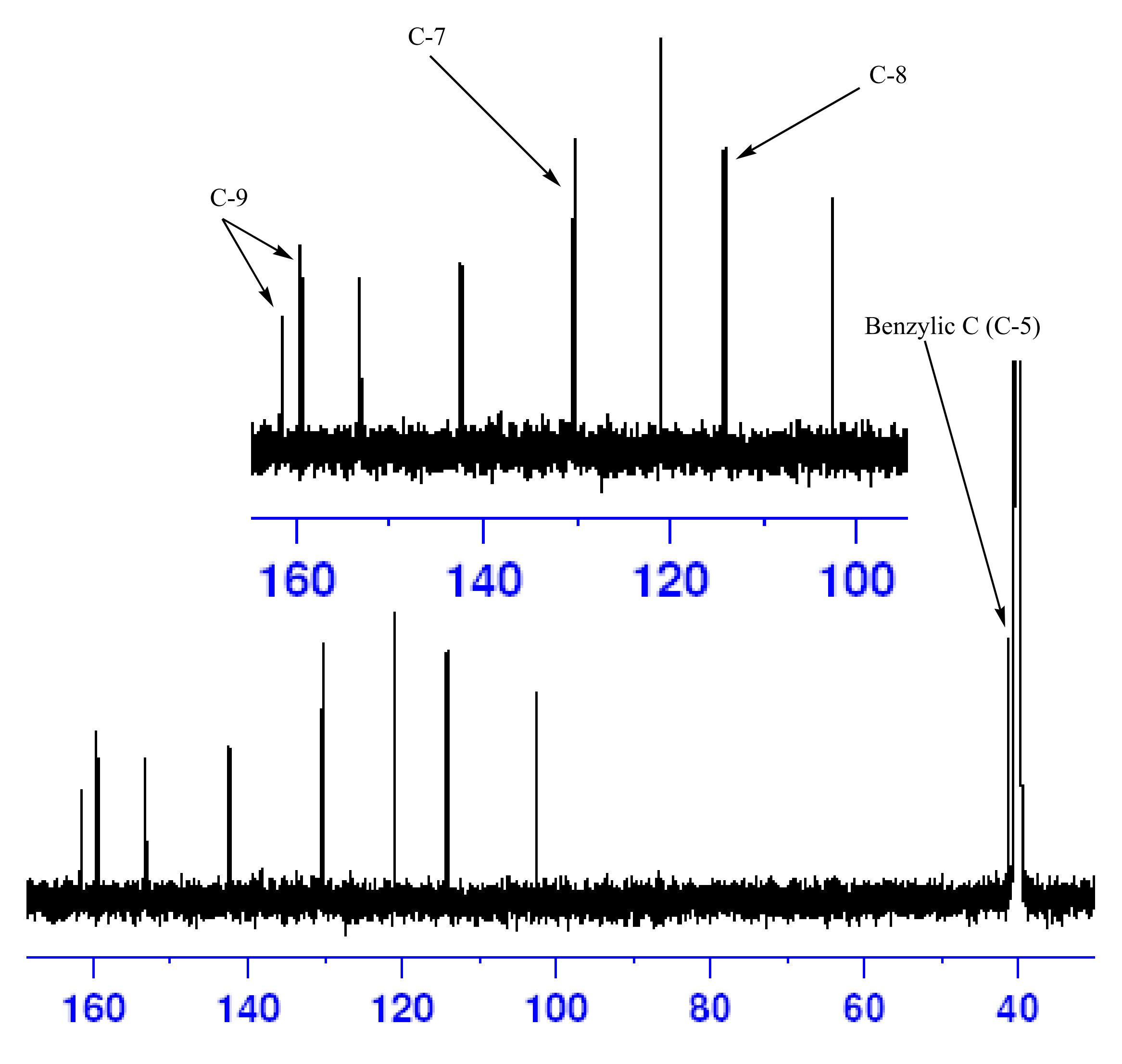


Figure (13): ^{13}C-NMR spectra for 51 ($C_{52}H_{36}F_4O_8$) compound in DMSO-d_6 as solvent and TMS as internal standard (125 MHz).

3.2.4 Crystal Structure of 49 and 51 Lattice Inclusion Compounds

3.2.4.1 X-ray quality single crystal preparation

The structure and function of biological molecules are to a large degree determined by hydrogen bonding. This is the case for proteins, nucleic acids, carbohydrates, membranes and also the aqueous medium in which these components are held.

Suitable single crystals for X-ray analyses were obtained by dissolving 30 mg of the titled compound in 1 mL of dimethylsulfoxide (DMSO). The solvent was allowed to stand and slowly evaporated at room temperature.

3.2.4.2 Supporting Information Available

Crystallographic data (cif) have been deposited with the Cambridge Structural Data Centre (CCDC) with reference numbers (915290-915291). See http://www.ccdc.cam.ac.uk/conts/retrieving.html for crystallographic data in cif or other electronic format. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: 44(0)-1223-336033 or Email: deposit@ccdc.cam.ac.uk.

3.2.4.3 Crystal structure of 49-DMSO lattice inclusion system

Compound **49** was crystallized from fresh sample of dimethylsulfoxide and led to inclusion crystals of (**49**).$(C_2H_6SO)_8$ in a triclinic system with P-1 space group. Table 6 illustrates the crystal data and structure refinements of compound **49** lattice inclusion system. Initial looking into the solid state structure of the above inclusion system revealed that the asymmetric unit contains one titled host molecule (**49**) and eight guest molecules (DMSO). Two of the eight DMSO guest molecules have some degree of disorder. The host adopted the chair conformer of the calix[4]arene derivative. In addition, there are a number of supramolecular noncovalent interactions such as H-bonding between the OH of the host molecule and the O and S atoms of the guest molecule; figure 14 represents framework atoms of **49** lattice inclusion host. Host-guest interactions are clearly existed in the crystal structure of 49-DMSO inclusion system i.e. chlorine atom (Cl8) of one host molecule is interacting with an oxygen atom (O3S) of a guest molecule with a contact distance of 3.23 Å. In addition, hydrogen atom (H8) of a hydroxy group of a host molecule is interacting with an oxygen atom (O2S) of another guest molecule with a contact distance of 1.84 Å (Figure 15), different types of hydrogen bond angles and lengths that existed in the crystal structure of **49**-DMSO lattice inclusion system are presented in table 7. Centrosymmetric dimmer was adopted by the crystal structure of **49** in which chlorine atom of one host is hydrogen bonded with hydrogen atom of another host molecule with bond distances of 2.67 Å and 2.78 Å as illustrated in figure 16. The presence of three hetero atoms in the crystal structure of **49** has led to very interesting intermolecular non-covalent interactions such as; S…S (3.54 Å), S…O (3.28 Å), S…Cl (3.90 Å), O…Cl (3.23 and 3.93 Å), Cl…Cl (3.95 Å) and O…O (3.85 and 3.75 Å). In addition, careful analyses for the crystal packing of **49** revealed

that sulfur and oxygen atoms of both host and guest are involved in network of intermolecular hydrogen bondings in terms of: host-guest (S…H-O-Ar, 2.87 Å), guest-guest ($(CH_3)_2OS$…H-CH_2-SO-CH_3, 2.96 Å), guest-guest ($(CH_3)_2SO$…H-CH_2-SO-CH_3, 2.57 Å), and host-guest ($(CH_3)_2SO$…H-O-Ar, 1.81 and 1.85 Å).

Table 6

Crystal data and structure refinement for 49-DMSO inclusion system.

CCDC deposit no.	915290	
Empirical formula	$C_{68} H_{84} Cl_4 O_{16} S_8$	
Formula weight	1555.63	
Temperature	223(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 13.0931(6) Å	α= 82.7770(10)°.
	b = 15.0868(7) Å	β= 79.5100(10)°.
	c = 20.9627(9) Å	γ = 70.7850(10)°.
Volume	3835.1(3) $Å^3$	
Z	2	
Density (calculated)	1.347 gm cm^{-3}	
Absorption coefficient	0.434 mm^{-1}	
F(000)	1632	
Crystal size	0.70 x 0.60 x 0.56 mm^3	
Theta range for data collection	1.68 to 27.50°.	
Index ranges	-16<=h<=17, -15<=k<=19, -27<=l<=24	
Reflections collected	27332	
Independent reflections	17523 [R(int) = 0.0181]	
Completeness to theta = 27.50°	99.4 %	
Absorption correction	Sadabs, (Sheldrick 2001)	
Max. and min. transmission	0.7931 and 0.7510	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	17523 / 16 / 900	
Goodness-of-fit on F^2	1.047	
Final R indices [I>2sigma(I)]	R1 = 0.0682, wR2 = 0.1847	
R indices (all data)	R1 = 0.0872, wR2 = 0.1990	
Largest diff. peak and hole	1.360 and -1.158 e.$Å^{-3}$	

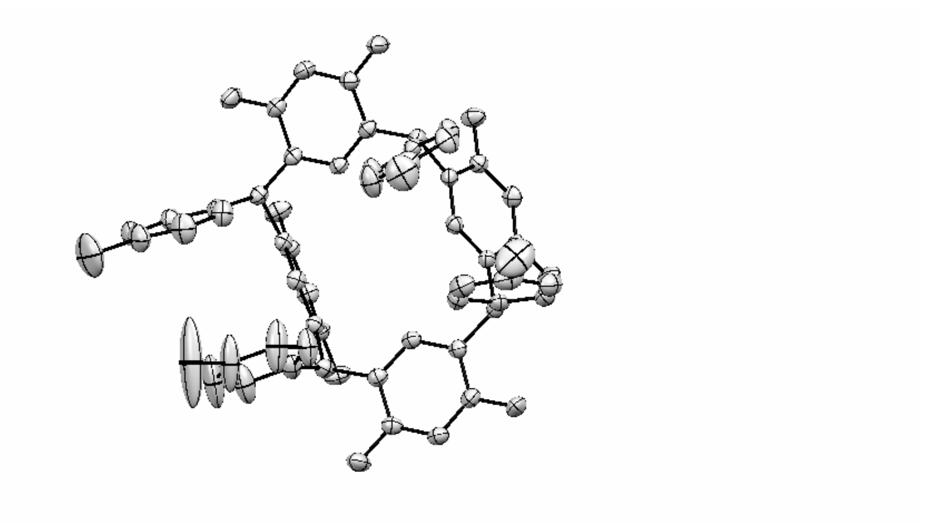


Figure (14)
Framework atoms of **49** lattice inclusion host, hydrogen atoms were omitted for clarity. Thermal *ellipses* are shown in 50% probability.

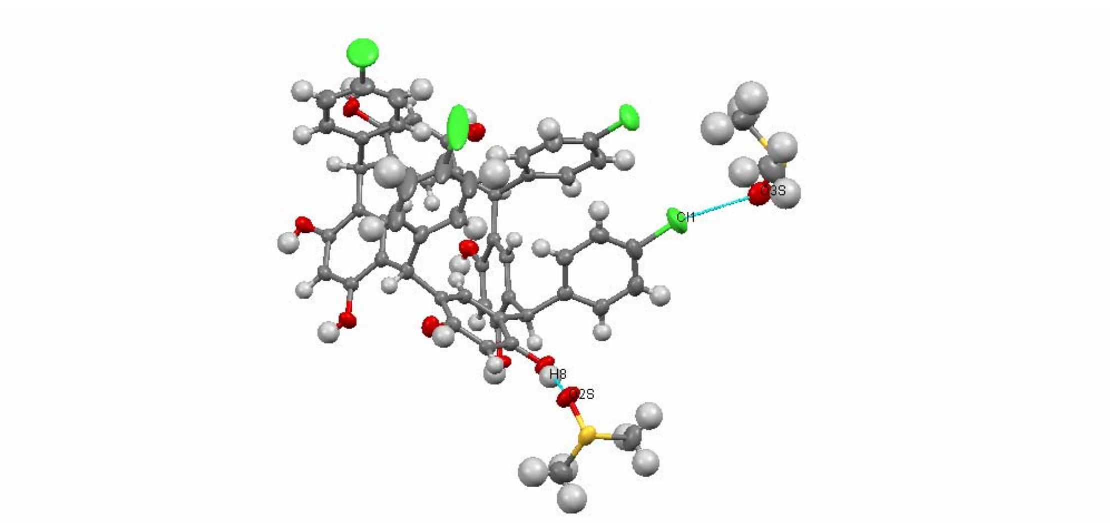


Figure (15)
Host-guest interactions between one host molecule and two guest molecules including Cl…O and H…O supramolecular interactions.

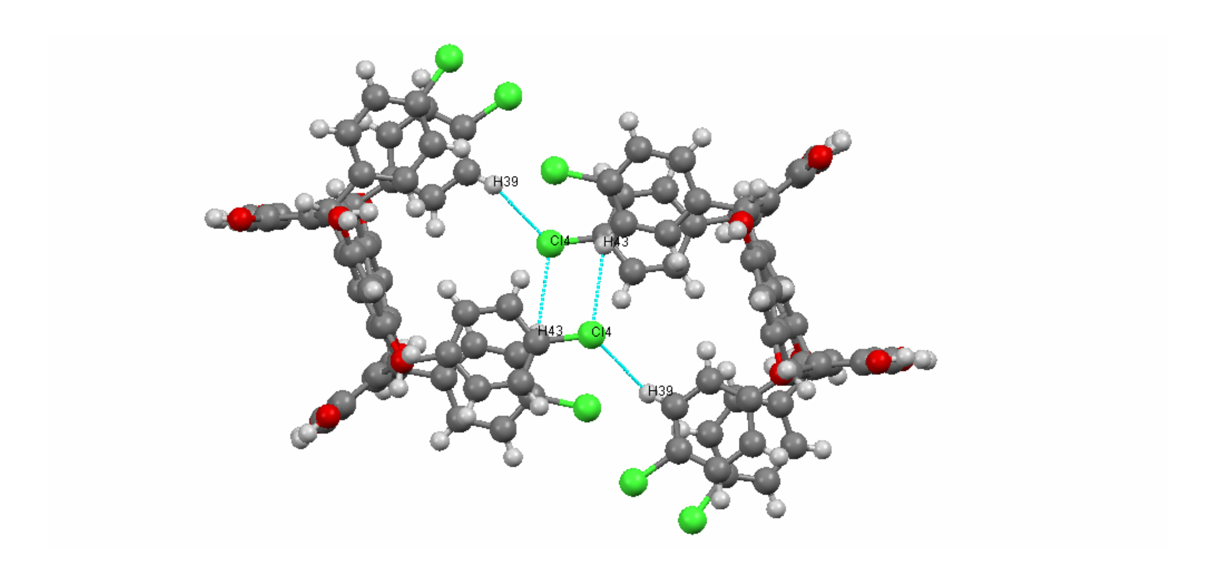


Figure (16)
Ar-Cl…H-Ar double interactions that formed centrosymmetric dimmer.

Table 7
Hydrogen bond lengths and angles for 49-DMSO lattice inclusion system [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(7S)#1	0.83	1.81	2.636(3)	173.4
O(2)-H(2)...O(8S)#1	0.83	1.82	2.622(9)	161.3
O(2)-H(2)...O(8SA)#1	0.83	1.88	2.663(6)	156.6
O(3)-H(3)...O(4S)#2	0.83	1.85	2.673(3)	169.3
O(4)-H(4)...O(6S)#3	0.83	1.87	2.695(3)	176.4
O(5)-H(5)...O(1S)	0.83	1.85	2.679(3)	174.4
O(6)-H(6)...O(5S)	0.83	1.81	2.611(3)	161.0
O(7)-H(7)...O(3S)#4	0.83	1.84	2.659(3)	167.0
O(8)-H(8)...O(2S)#5	0.83	1.84	2.666(3)	171.8

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y+1,-z+1 #2 x+1,y,z #3 -x+1,-y+1,-z #4 x-1,y+1,z #5 -x+1,-y+2,-z+1

3.2.4.4 Crystal structure of 51-DMSO lattice inclusion system

Compound **51** was crystallized from fresh sample of dimethylsulfoxide and led to inclusion crystals of (**51**).$(C_2H_6SO)_8$in a triclinic system with P-1 space group. Table 8 illustrates the crystal data and structure refinements of compound **51** lattice inclusion system. Initial looking into the solid state structure of the above inclusion system revealed that the asymmetric unit contains one titled host molecule (**51**) and eight guest molecules (DMSO). Two of the eight DMSO guest molecules have some degree of disorder (flipping over the S atom). Unlike **49**, **51** host molecule adopted the cone conformer of the calix[4]arene derivative. In addition, there are a number of supramolecular noncovalent interactions such as H-bonding between the OH of the host molecule and the O and S atoms of the guest molecule, figure 17 represents a framework atoms of **51** lattice inclusion host. Host-guest interactions are clearly existed in the crystal structure of **51**-DMSO inclusion system i.e. hydrogen atom (H2) of hydroxy group of one host molecule is interacting with an oxygen atom (O3S) of a guest molecule with a contact distance of 1.88 Å. In addition, hydrogen atom (H1) of a hydroxy group of same host molecule is interacting with an oxygen atom (O2S) of another guest molecule with a contact distance of 1.86 Å (Figure 18), different types of hydrogen bond angles and lengths that existed in the crystal structure of **51**-DMSO lattice inclusion system are presented in table 9. Sulfur atom of the guest molecule is interacting with hydrogen atom of host molecule with bond distances of 2.71 and 2.82 Å, no sulfur interaction with another guest molecule is detected in the crystal packing of **51**. Bifurcated oxygen interaction between oxygen atom of one guest molecule with host molecule with bond distances of 2.60 and 1.85 Å (Figure 19). Furthermore, crystal packing of **51** proved that two DMSO guest molecules are intermolecularly hydrogen-bonded with host molecule with bond distances of 1.85, 1.88, and 2.61 Å as illustrated in figure 20. Fluorine atom of the host molecule is interacted with hydrogen atom of guest molecule ((CH_3SOCH_2-H…F-Ar, 2.47 Å) as shown in figure 21. Other hetero atoms exist in the molecular structure of both host and guest are interacting via different motives such as; Ar-F…F-Ar (3.48 Å), Ar-F…O=$S(CH_3)_2$ (4.15, 4.42, 4.79, 4.76 Å), Ar-F…$SO(CH_3)_2$ (3.85 and 3.58 Å), S…S (3.6 Å).

Table 8.

Crystal data and structure refinement for 51-DMSO lattice inclusion system.

CCDC deposit no.	915291	
Empirical formula	$C_{68} H_{84} F_4 O_{16} S_8$	
Formula weight	1489.83	
Temperature	223(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.2575(5) Å	α= 105.1780(10)°.
	b = 13.9642(7) Å	β= 107.1530(10)°.
	c = 15.3376(8) Å	γ = 107.2570(10)°.
Volume	1852.01(16) $Å^3$	
Z	1	
Density (calculated)	1.336 gm cm^{-3}	
Absorption coefficient	0.314 mm^{-1}	
F(000)	784	
Crystal size	0.30 x 0.26 x 0.10 mm^3	
Theta range for data collection	1.50 to 25.00°.	
Index ranges	-12<=h<=12, -16<=k<=16, -18<=l<=18	
Reflections collected	20132	
Independent reflections	6540 [R(int) = 0.0469]	
Completeness to theta = 25.00°	100.0 %	
Absorption correction	Sadabs, (Sheldrick 2001)	
Max. and min. transmission	0.9693 and 0.9117	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6540 / 0 / 449	
Goodness-of-fit on F^2	1.147	
Final R indices [I>2sigma(I)]	R1 = 0.0799, wR2 = 0.1693	
R indices (all data)	R1 = 0.1034, wR2 = 0.1802	
Largest diff. peak and hole	0.463 and -0.587 e.$Å^{-3}$	

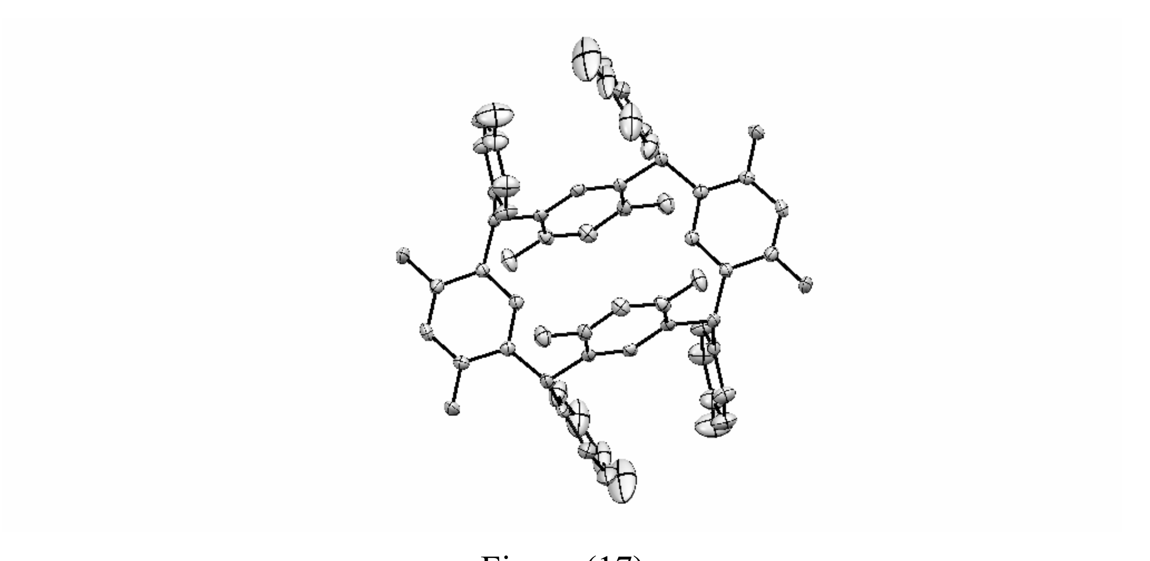


Figure (17)
Framework atoms of **51** lattice inclusion host, hydrogen atoms were omitted for clarity. Thermal *ellipses* are shown in 50% probability.

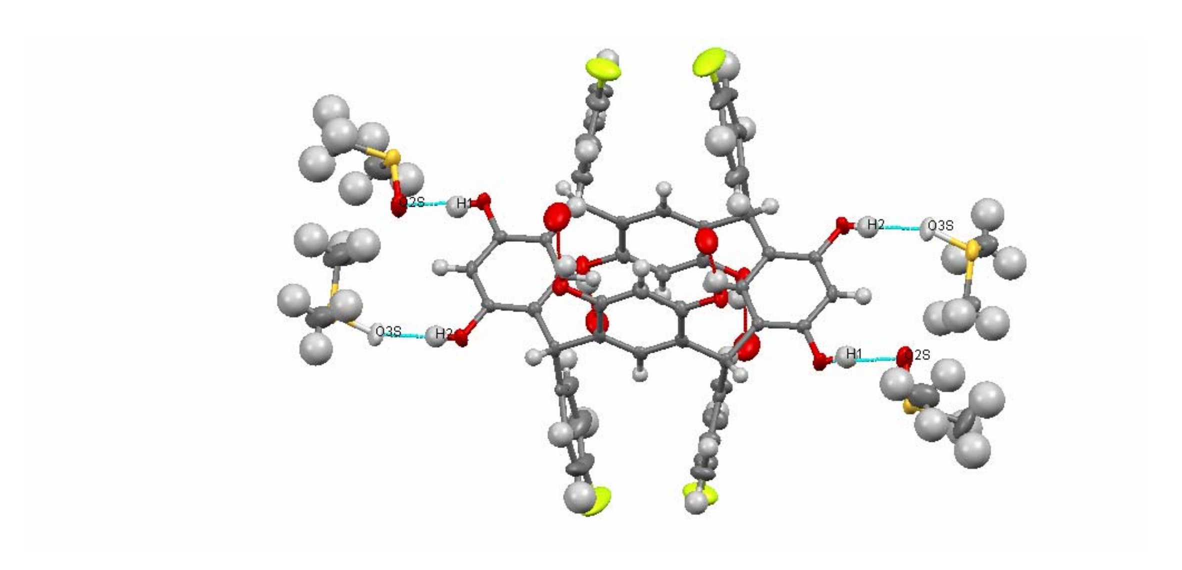


Figure (18)
Host-guest interactions between one host molecule and four guest molecules including H…O supramolecular interactions.

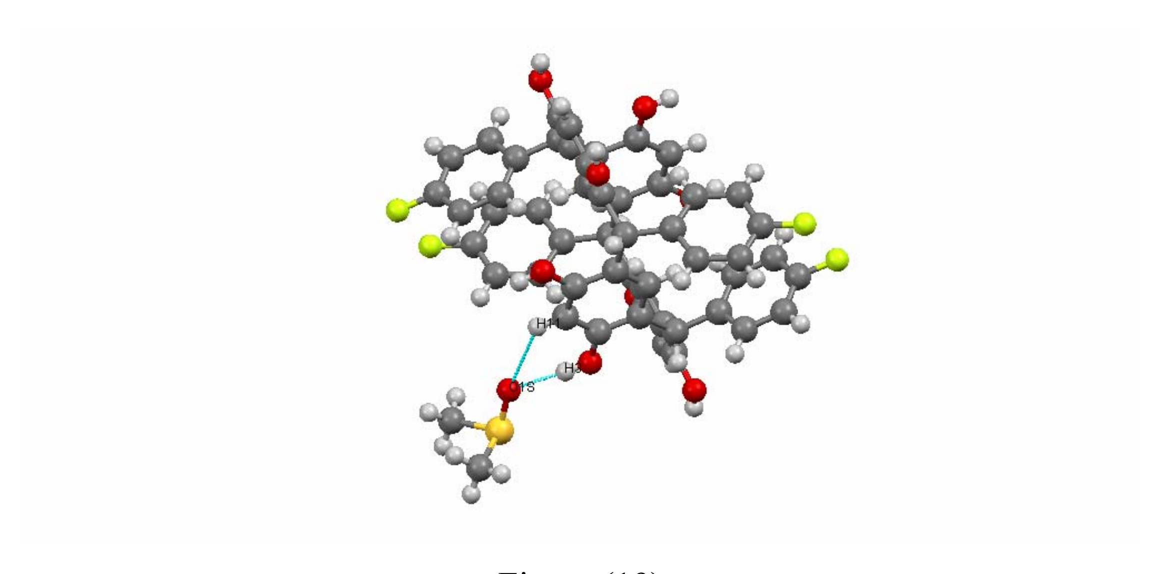


Figure (19)
Bifurcated host-guest interactions with O1S…H11 and O1S…H3 bond distances of 2.60 and 1.85, respectively.

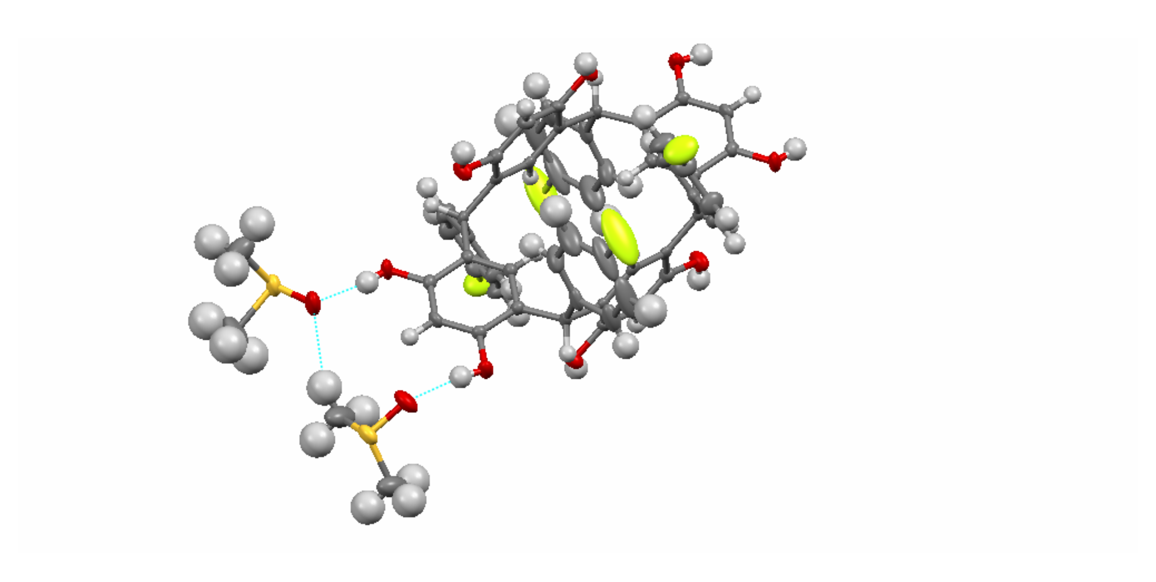


Figure (20)
Different guest-guest-host intermolecular motives exist in crystal structure of **51**.

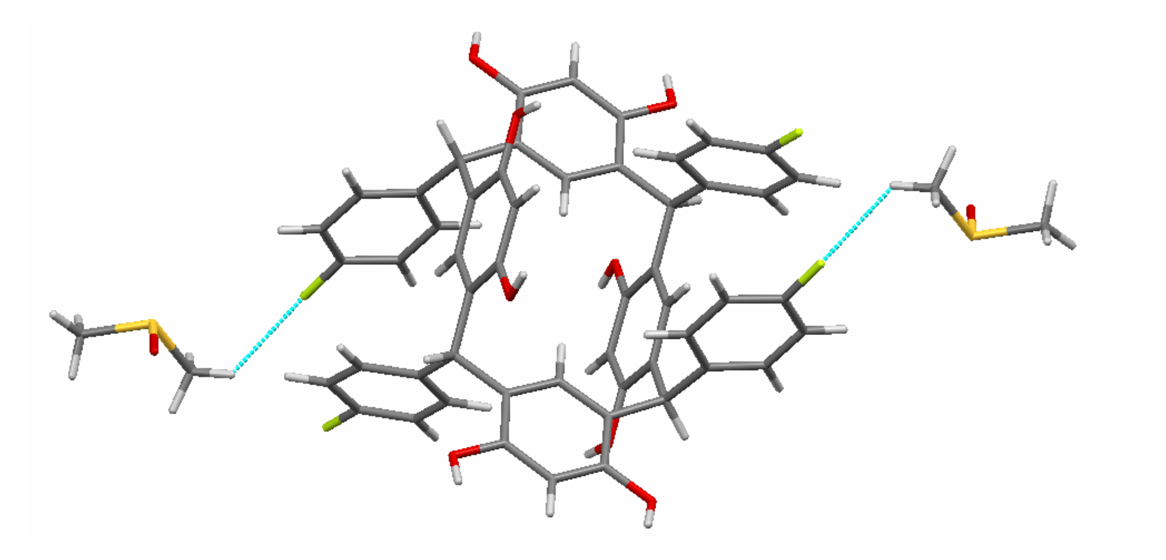


Figure (21) Host-guest interaction in which fluorine atom of one host molecule is interacting with hydrogen atom of guest molecule.

Table 9

Hydrogen bonds lengths and angles for 51-DMSO lattice inclusion system [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1)-H(1)...O(2S)#2	0.83	1.85	2.669(4)	166.7
O(2)-H(2)...O(3S)	0.83	1.88	2.706(4)	172.8
O(3)-H(3)...O(1S)#3	0.83	1.85	2.677(4)	175.8
O(4)-H(4)...O(4S)#4	0.83	1.91	2.714(5)	162.8

Symmetry transformations used to generate equivalent atoms:

#1 -x+1,-y+1,-z+2 #2 x-1,y,z #3 x,y+1,z+1 #4 x,y,z+1

3.2.5 In Vitro Antimicrobial Activity

As shown in Tables.10 and 11, gram negative bacteria were resistant to the applied compounds that showed a weak to moderate activity against the tested gram positive ones. In agar diffusion test the microorganism were susceptible at concentration started from 0.5 mg/disc with the fluorinated calixarene derivative being more effective that the chlor- and bromo as well as methoxy-derivatives.

Despite the concentrations required from synthesized compounds in the agar diffusion test to affect the growth of tested bacteria, they were effective at MIC-values 2-10 fold lower than that in agar diffusion assay. Moreover, the brominated derivative was more potent that the other calixarenes with MIC ranged between 15.6-125 µg/mL. The differences in activity in both antimicrobial assays may be attributed to their low solubility in water that renders their diffusion in agar plates. Such observation was noticed previously by Salem et al., (2001) and agreed with what stated in Mokhtari & Pourabdollah,(2012). In addition the resistance of Gram negative bacteria tested compounds could be due to the low permeability of the outer membrane of these bacteria to the applied calixarene derivatives.

Table 10
Inhibition zone (mm) caused by synthesized compounds in agar diffusion test (Conc. 0.5/1 mg/disc)

Organisms	Inhibition Zone (mm)			
	49	50	51	52
Bacillus subtilis	7/7	7/7	8/8	-/8
Staphylococcus aureus	7/7	7/8	10/11	-/8
Micrococcus luteus	8/8	9/10	9/10	9/10

Table 11
Minimal inhibitory concentration (MIC) of the synthesized compounds in the serial dilution assay

Organisms	MIC (µg/mL)				
	49	50	51	52	Chl.
Bacillus subtilis	62.5	125	500	125	<0.8
Staphylococcus aureus	125	62.5	125	500	<0.8
Micrococcus luteus	31.2	15.6	62.5	62.5	<0.8

Chl. : Chloramphenicol

3.3 Conclusions

New Calixarene host molecules derived from reaction of resorcinol in a mixture of ethanol and catalytic amount of concentrated HCl with four different of para-substituted benzaldehyde solution namely, 4-cloro-bezaldehyde, 4-bromobezaldehyde, 4-florobezaldehyde, and 4-methoxy-benzaldehyde by condensation refluxed method have been prepared. The proposed structure of these new compounds are shown in scheme (11). The new compounds are expected to have good applications in biological and medical fields due to their potential activity.

The structure of 49 and 51 lattice inclusion compounds have been determined by single crystal X-ray diffraction. The non-covalent supramolecular chemistry involved in the crystal structure of these inclusion compounds have been carefully investigated. In crystal engineering and supramolecular chemistry; understanding the intermolecular forces that involved in any crystal structure is considered to be very useful way to predict the physical properties of it. The structure and function of biological molecules are to a large degree determined by hydrogen bonding. This is the case for proteins, nucleic acids, carbohydrates, membranes and also the aqueous medium in which these components are held.

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APPENDIX I
SPECTRA

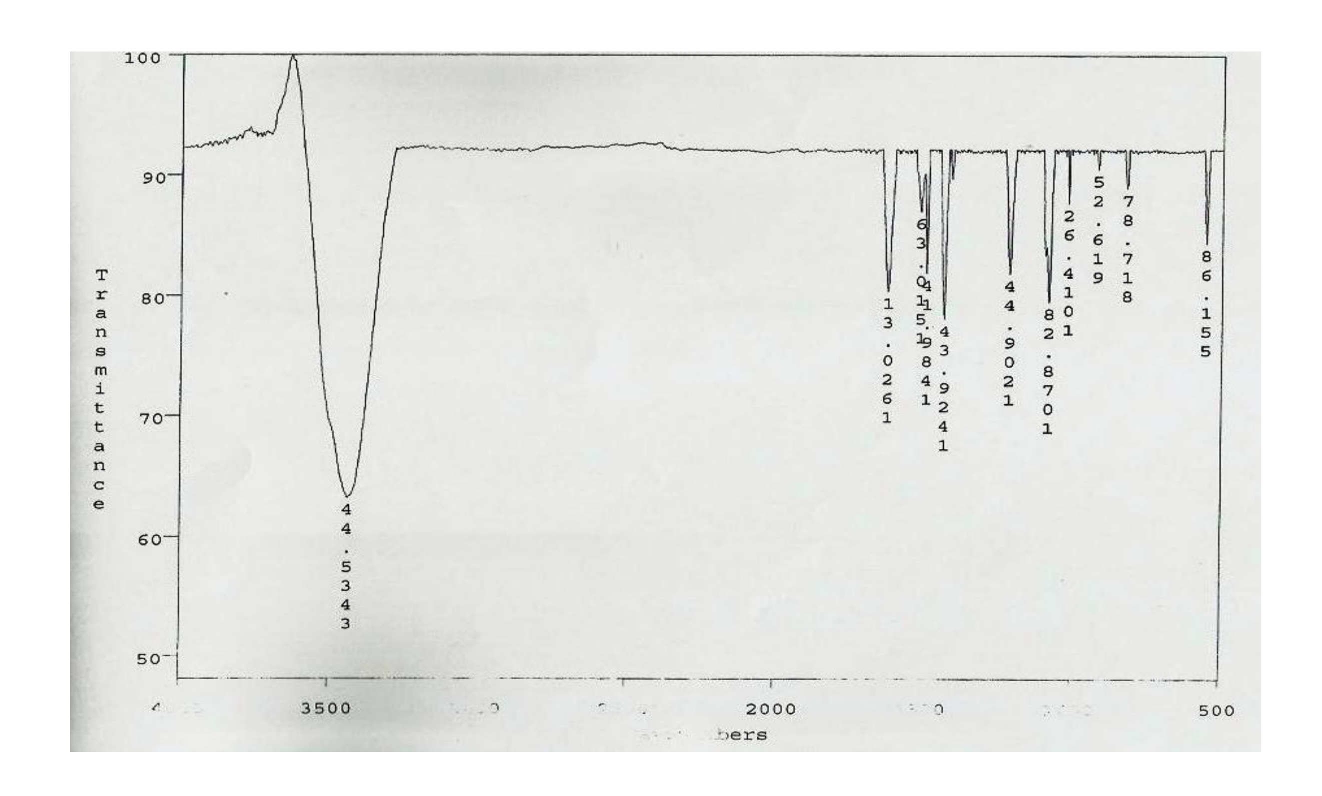


Figure 22. FTIR spectra of 49 compound.

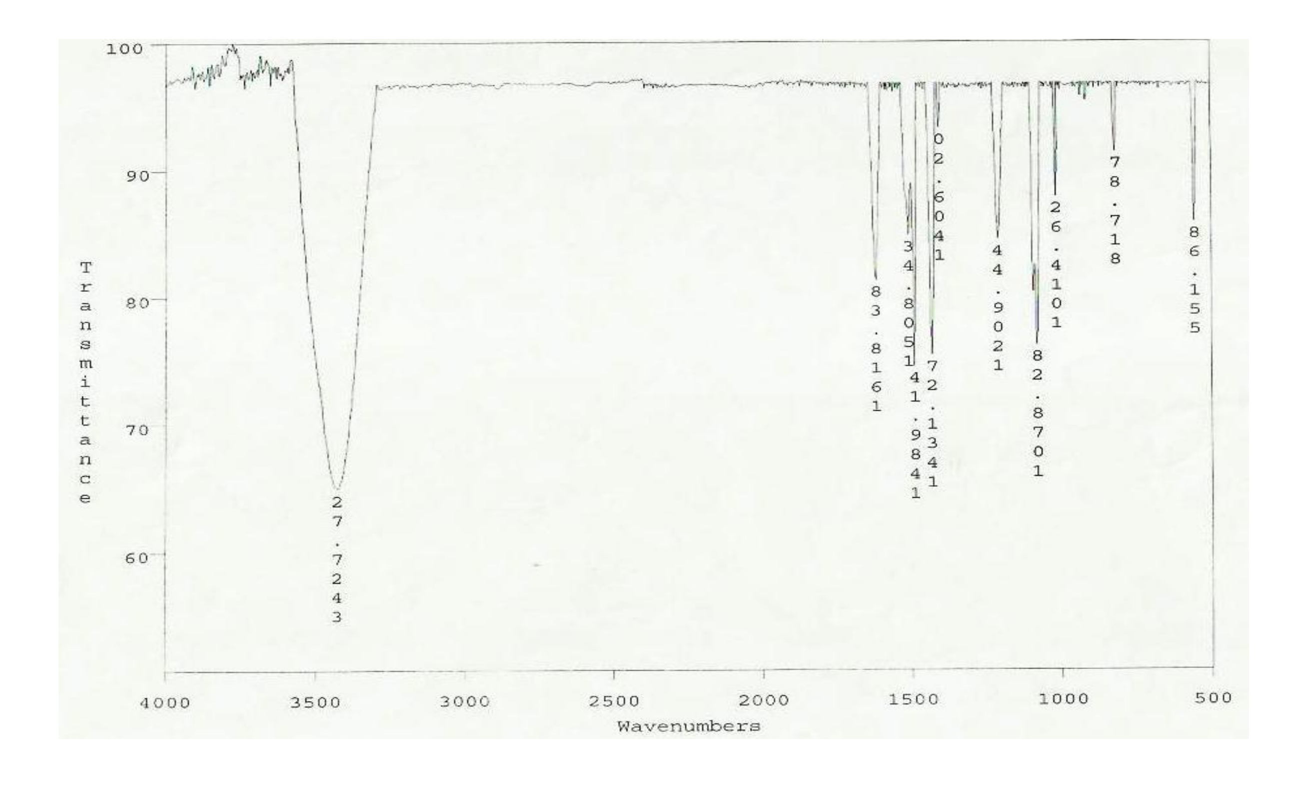


Figure 23. FTIR spectra of 49 compound in Cyclopentanolas solvent

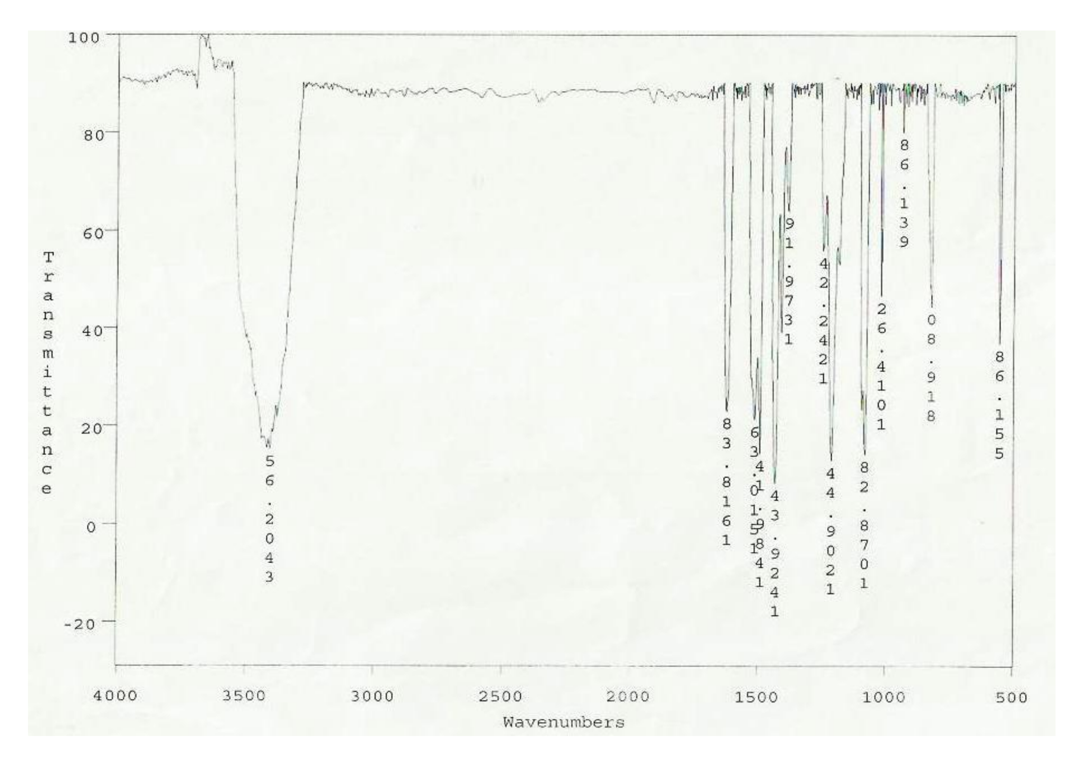


Figure 24. FTIR spectra of 49 compound in Ethanol as solvent

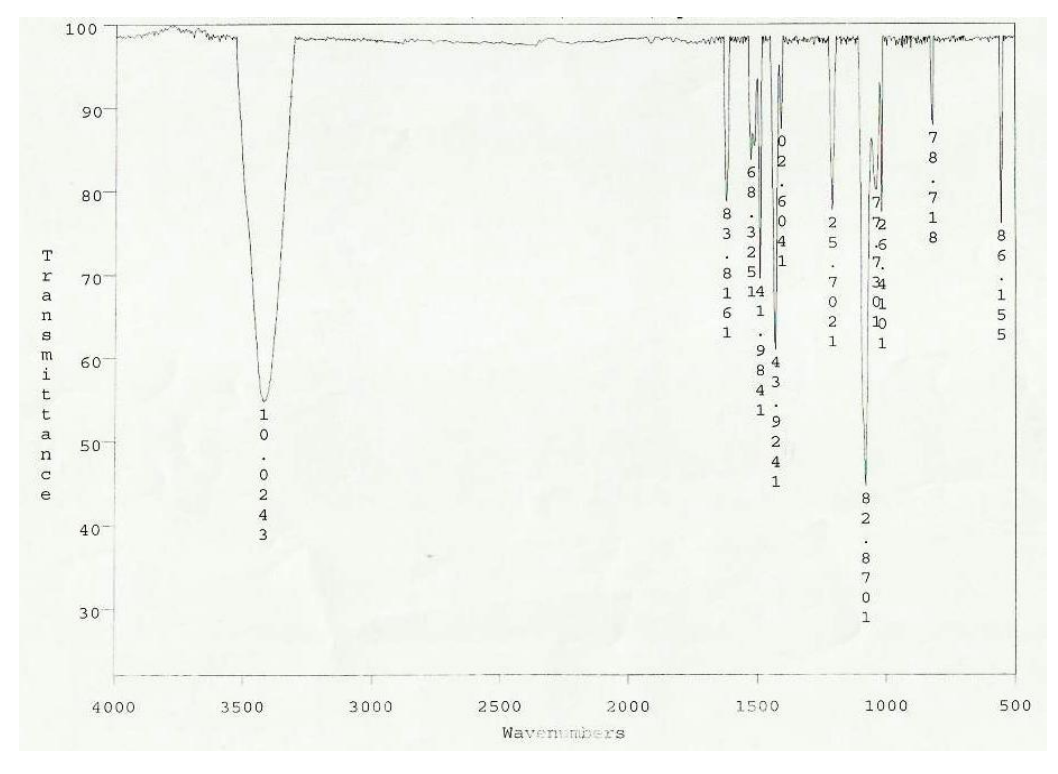


Figure 25. FTIR spectra of 49 compound in Ethyleneglycol as solvent

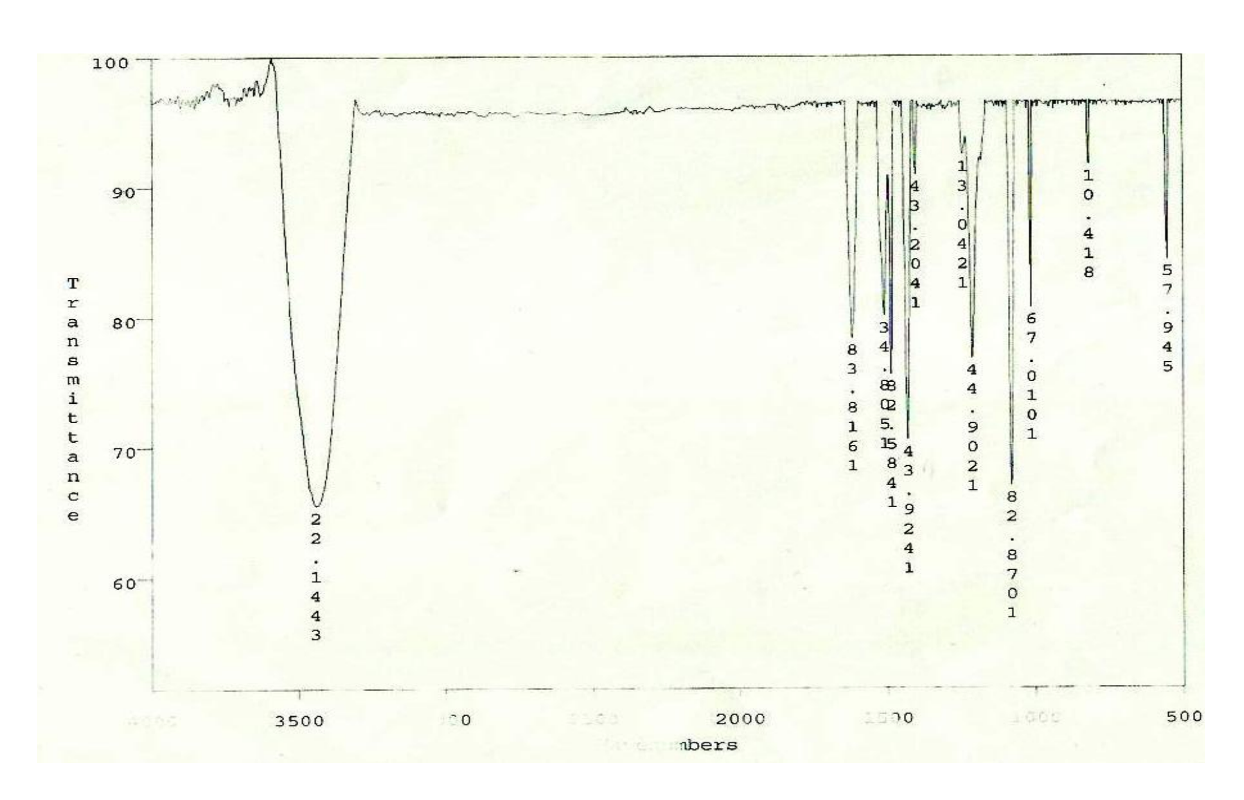


Figure 26. FTIR spectra of 50 compound

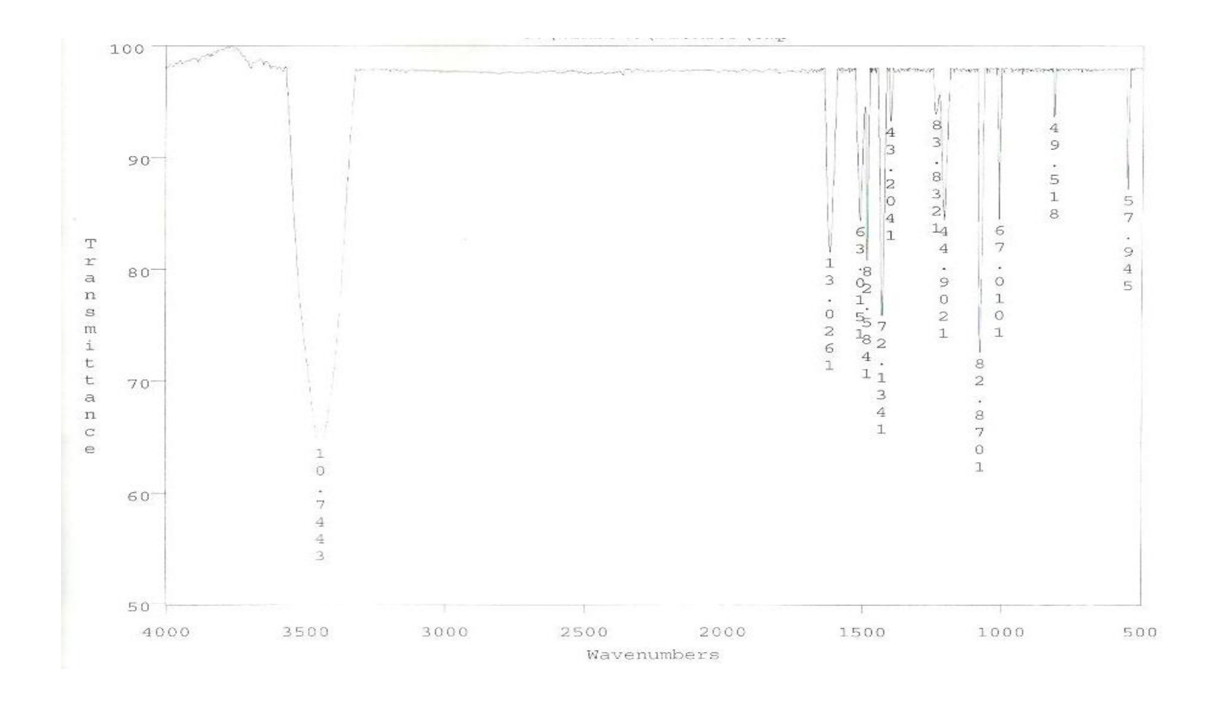


Figure 27. FTIR spectra of 50 compound in Cyclopentanol as solvent

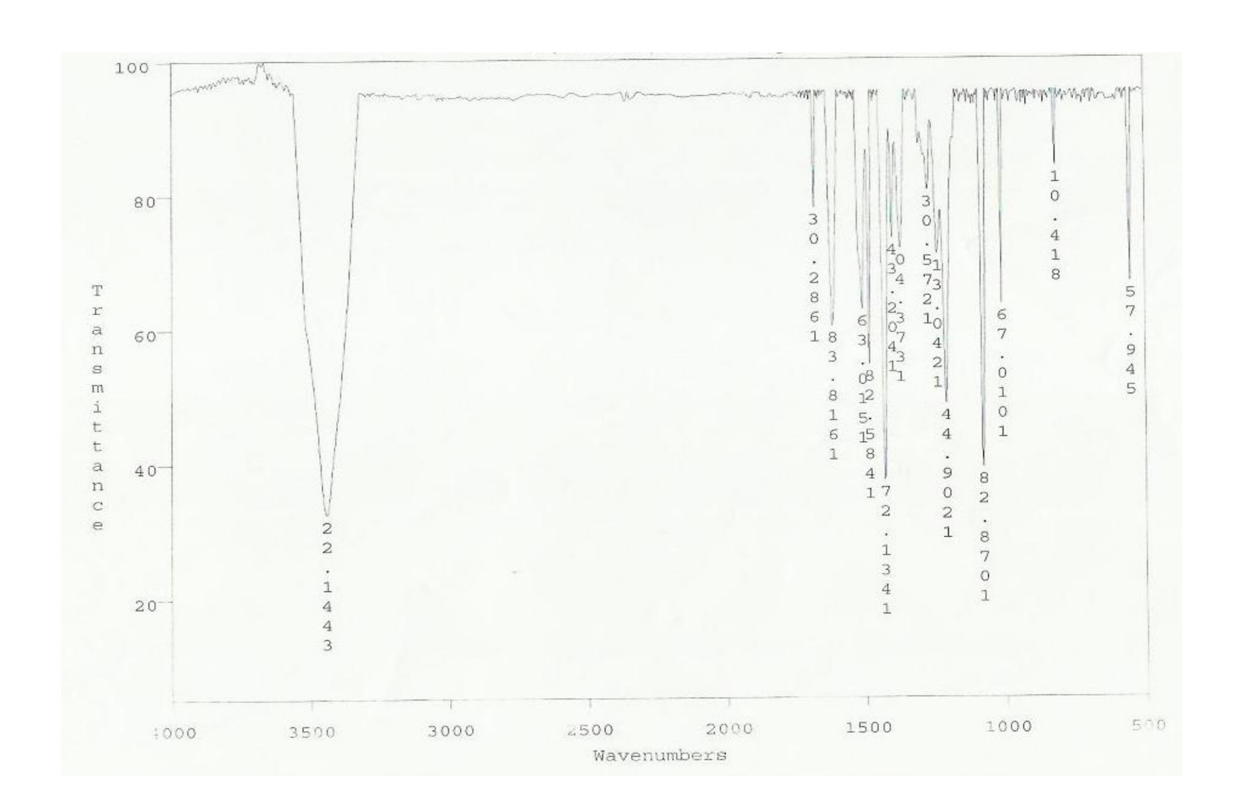


Figure 28. FTIR spectra of 50 compound in Ethanol as solvent

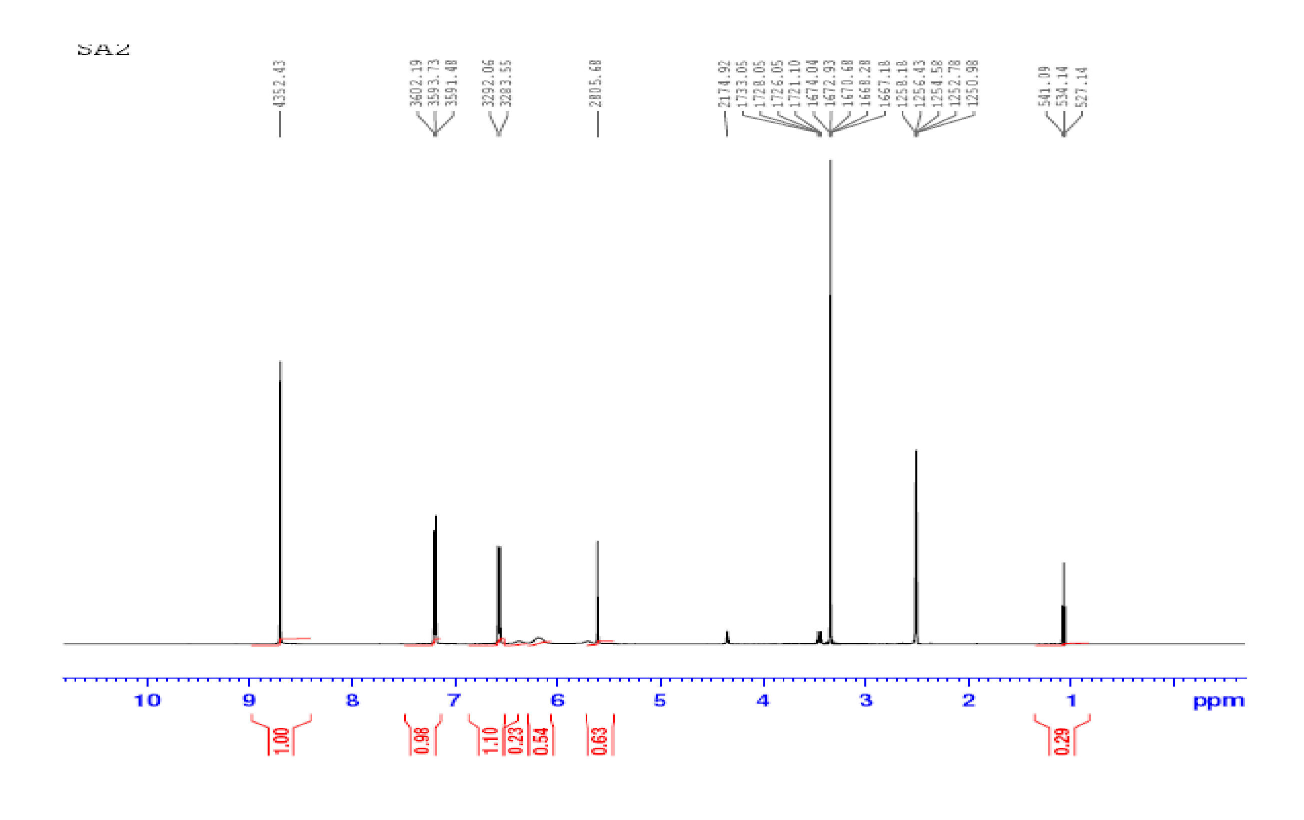


Figure 29. ^{1}H-NMR spectrum for 50 compound.

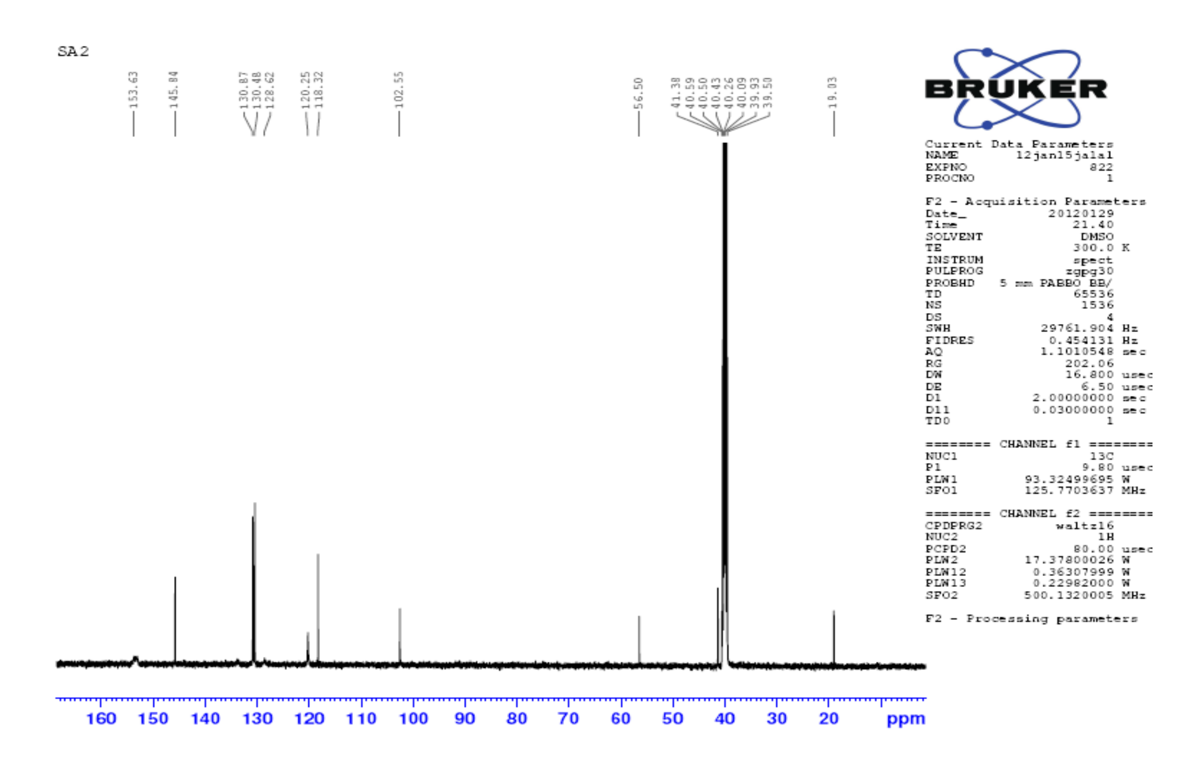


Figure 30. ^{13}C-NMR spectrum for 50 compound.

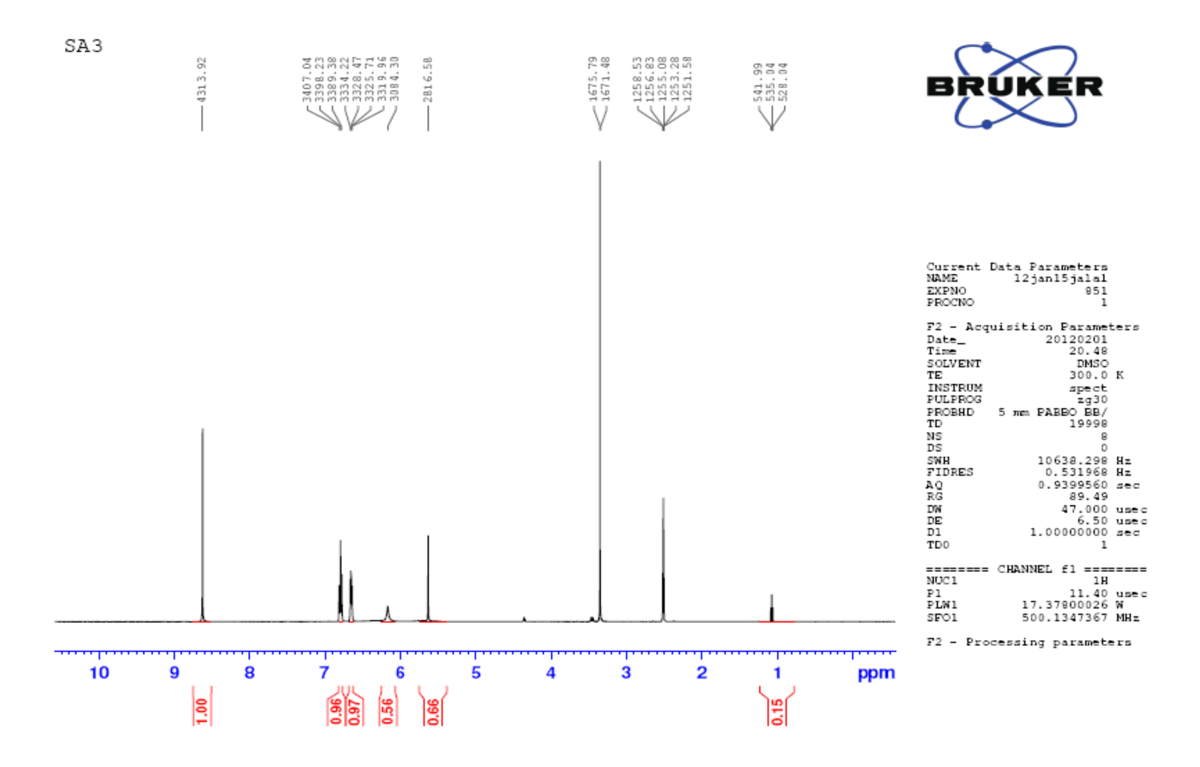


Figure 31. ^{1}H-NMR spectrum for 51 compound.

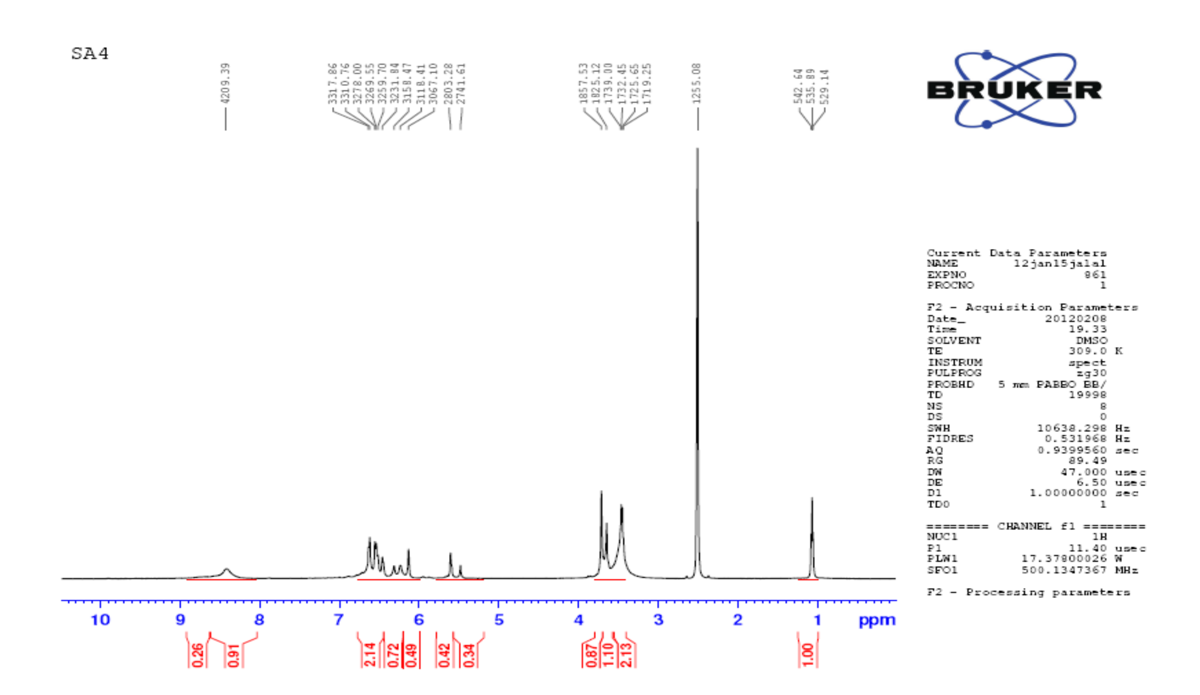


Figure 32. ^{1}H-NMR spectrum for 52 compound.

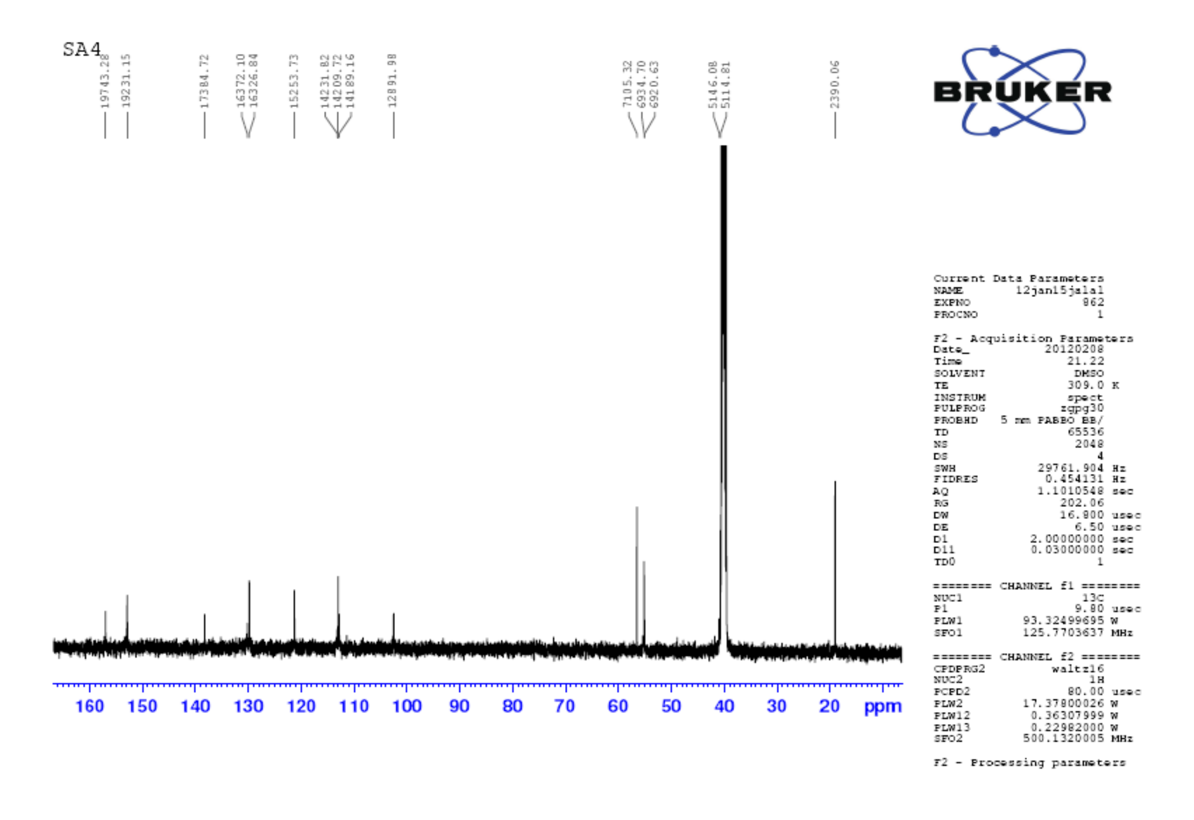


Figure 33. ^{13}C-NMR spectrum for 52 compound